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In 1927 the writer decided to carry out experiments on the artificial inoculation of cotton plants with the organism under controlled environmental conditions. After some early unsuccessful attempts, the following apparatus was designed and proved satisfactory.

Description of infection chamber.

The apparatus (Fig. 1) is essentially a double-walled infection chamber suitably insulated against heat-loss and provided with means for maintaining the air temperature and humidity within it at any

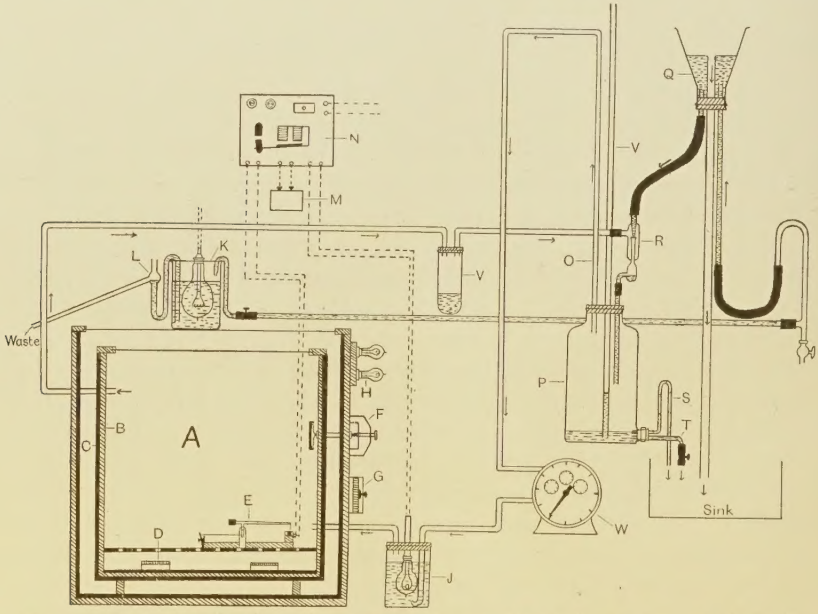


Fig. 1. *A*, infection chamber; *B*, wooden walls (cross hatched); *C*, asbestos linings; *D*, heating units; *E*, hygrostat; *F*, temperature switch; *G*, variable resistance; *H*, resistance lamps; *J*, conditioning vessel; *K*, lamp for illumination; *L*, constant-level overflow; *M*, battery; *N*, relay; *O*, air tube; *P*, aspirator; *Q*, glass funnel; *R*, filter pump; *S*, regulating outflow; *T*, constant outflow; *V*, trap for condensed water; *W*, air meter.

required degree. The chamber is made by fixing a wooden box (in this case a tea chest 20 in. × 20 in. floor space) on blocks within a larger packing-case so as to leave a dead-air space of about two inches all round between the boxes. The inner walls and floor of the outer case and the outside of the inner chest are covered with "Uralite" asbestos-

board as lagging, and each box is provided with a hinged glass lid. On the floor of the inner case is another square of asbestos board carrying the four heating units, which in this case were small wire-wound mica "strip-heaters" as used in small electric incubators. These are held in position by brass clips screwed to the asbestos, and the electrical connections are made to the clips. An inch above the heating units is a false bottom also made of "Uralite" and pierced by a large number of holes; this serves to distribute the heat more uniformly. On the front wall of the chamber is fixed a Hearson Electric Temperature Switch (*F*), the

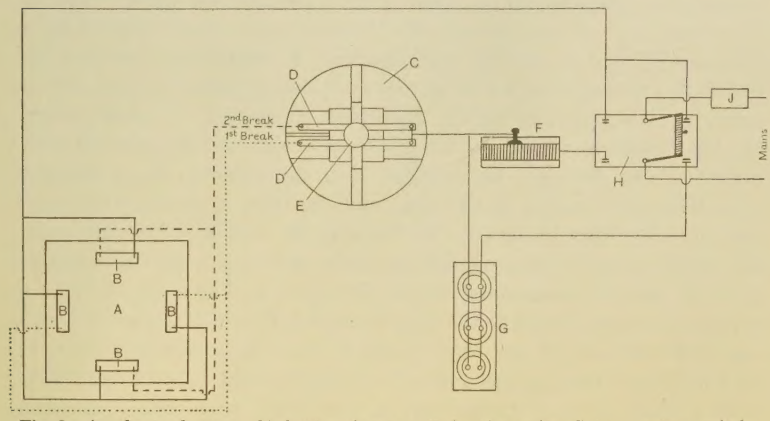


Fig. 2. *A*, asbestos bottom of infection chamber; *B*, heating units; *C*, temperature switch; *D*, spring contacts; *E*, fibre disc; *F*, variable resistance; *G*, resistance lamps; *H*, switch; *J*, fuse.

brass sleeve of which passes through both walls of the chamber and is secured by lock-nuts. A suitable capsule placed in the brass stirrup controls the switch by means of the copper rod pressing against the fibre disc (*E*, Fig. 2) through which the two spring contacts (*D*, Fig. 2) pass. The two contacts are each connected to two of the heating units wired in parallel, the free ends of the strip heaters being all connected to one of the leads from the 100-volt mains. A two-pole, double-throw switch (*H*, Fig. 2) allows either a variable 40 ohm resistance (for the higher temperatures—above 30° C.) or one or more carbon-filament lamps in parallel (for the lower temperatures) to be connected in series with the other lead and the remaining terminal of the switch. By this means the current can be controlled to a suitable degree to ensure that it shall be on and off for approximately the same length of time, this

condition avoiding too rapid heating or cooling with the resulting wide fluctuations in temperature. A fuse in one main lead is provided for greater safety. The whole circuit is shown diagrammatically in Fig. 2.

The control of humidity presented greater difficulties, but finally the apparatus figured was found to be satisfactory. A glass vessel (*J*, Fig. 1) containing water (or for low humidities dilute sulphuric acid—about 30 per cent.), in which a carbon filament lamp is immersed, is provided with an inlet tube drawn out to a small aperture and reaching nearly to the bottom of the vessel, and an outlet tube bent at right angles and passing through holes in the walls of the cases into the inner chamber. The short piece of tube outside the chamber is constantly heated by a very small flame to prevent condensation. A continuous stream of air is bubbled through this water by means of the apparatus figured, which embodies the principle of the Shenstone apparatus. A glass funnel (*Q*, Fig. 1) (a conical flask with the bottom broken out) is provided with an inlet from the main water-supply and two outlets, one, a straight wide-bore tube leading to the sink, and the other a smaller tube connected to the filter-pump (*R*). By keeping the supply sufficiently rapid for water always to be overflowing down the large outlet a constant head of water is maintained on the filter-pump. The latter delivers a mixture of air and water to the large aspirator (*P*) which is provided with one outlet for air (*O*) and two for water, one a siphon-shaped tube (*S*) of fairly small bore, and the other a wider, straight tube (*T*) provided with a short piece of rubber tubing controlled by a screw-clip. The second of these allows of an approximate adjustment of the outflow, after which the first acts as an automatic regulator in the following manner. There being a constant resistance to the passage of air through the vessel (*J*, Fig. 1), any increase of pressure in the aspirator will result in a faster stream of water through outlet (*S*) and the escape of a little air. For greater safety still the outlet for air (*O*) is carried up to a level higher than the funnel (*Q*) so that in the event of the outflow of water becoming blocked no water can be carried over into the chamber. A long straight tube (*V*) reaching to the bottom of the aspirator serves as a pressure gauge. The apparatus delivers approximately 1 cu. ft. of air per hour to the infection chamber, as measured by the air meter (*W*, Fig. 1).

The hair "hygrostat," which is shown in side view at *E*, Fig. 1, and in plan in Fig. 3, consists of a wooden base about eight inches long and two wide, at one end of which are screwed, one on top of the other, two uprights cut from stout copper or brass sheet. The outer and longer of these carries a screw which presses against the shorter and serves to

adjust the longer for purposes which will be apparent. A bundle of human hairs (*N*, Fig. 3) is connected at one end to this longer upright by passing through a small hole bored in the copper, a knot and short piece of stick preventing it from slipping through, and at the other to a short lever fixed to a horizontal axle (*L*, Fig. 3) pivoted on two more copper uprights screwed to the sides of the wooden base. The other end of the lever carries a long arm (in the actual apparatus the lever and arm were cut out of one piece of sheet tin) bearing at its end a piece

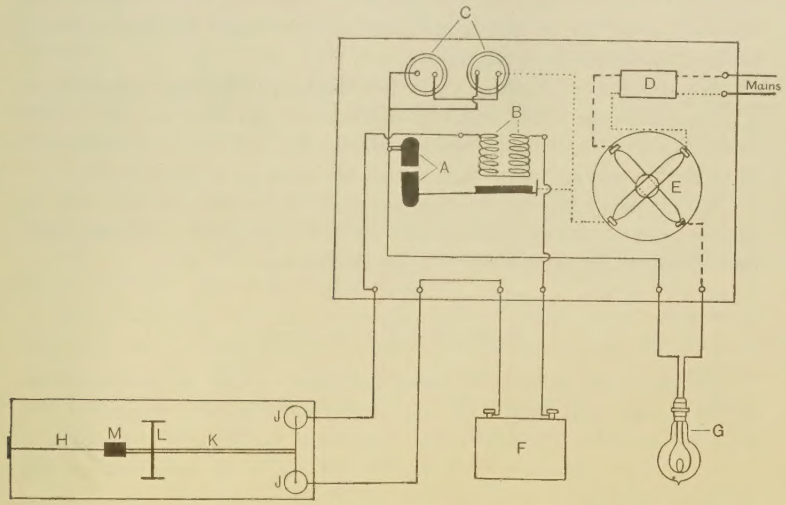


Fig. 3. *A*, carbon contacts; *B*, coils; *C*, lamp-holders (not used in this case); *D*, fuse; *E*, switch; *F*, 4-volt battery; *G*, heating lamp for conditioning vessel; *H*, hairs; *J*, mercury cups; *K*, lever; *L*, axle; *M*, counterpoise.

of platinum wire bent twice at right angles, the ends dipping into mercury cups (*J*, Fig. 3) made by sealing short pieces of glass tubing into holes in a block of wood by means of sealing wax. Pieces of thick copper wire passing through the wooden block make contact with the mercury. The cups are by this means connected with a carbon-contact relay (made by Messrs Gallenkamp and Co.) which switches on the current to the lamp, thus heating the water and causing the air entering the chamber to be charged with more water-vapour. When the humidity within the chamber rises to the required degree, the elongation of the hairs allows the long pointer to rise under the influence of the counterpoise (*M*, Fig. 3) and the relay current is interrupted at the mercury

cups. The hygrostat can be set for any required humidity by means of the adjusting screw referred to above. A diagram of the relay, hygrostat, and connections to the apparatus is given in Fig. 3.

A constant (though very slow) circulation of the air within the chamber is obtained by connecting the intake of the filter pump (*R*, Fig. 1) to a glass tube passing through the walls near the top of the inner chamber. Dishes containing calcium chloride are placed within the chamber so that the humidity is constantly tending to fall and is as constantly raised again by the entry of moist air. This is necessary, since otherwise the air within the chamber would soon become saturated by the transpiration of the plants.

Illumination is provided by a 400 candle-power lamp immersed in a beaker through which water is continuously flowing, the level being maintained by the constant-level overflow (*L*). It was found that this illumination was sufficient to maintain the stomata slightly open.

With this apparatus the temperature within the chamber can be kept constant to within half a degree on either side of the required point, and the humidity within a 4 to 5 per cent. range.

Description of experiments.

Young cotton plants of strains previously found to be susceptible to the disease were used. At first several varieties were employed, but later 1–2-months-old plants of the Ashmouni variety only were used.

A strong suspension of a young culture of *B. malvacearum* was prepared by shaking up seven loopfuls of the culture with about 20 c.c. of distilled water (a definitely turbid suspension), and the plants thoroughly sprayed with this by means of a nasal atomiser. The strain of the organism used was one isolated in the laboratory from infected seed kindly supplied by Mr R. E. Massey, Mycologist to the Sudan Government. The sprayed plants were then immediately placed in the infection chamber, where they remained for 48 hours, this period having been found to be sufficient for infection to take place. At the end of this period the plants were returned to the glasshouse and examined at intervals. In those cases where infection had occurred, very small water-soaked spots on the leaves were visible after a period varying from two to four weeks. Owing to the fluctuating conditions in the glasshouse during the season it was not thought worth while to record the exact incubation period.

Results of experiments.

The date given is that on which the plants were sprayed and placed in the chamber. In every case 48 hrs. was the period allowed in the apparatus.

Exp. 1. July 4th. 2 plants, Acala variety. Temp. 35–36° C. Humidity 83–87 per cent. No infection.

Exp. 2. July 12th. 1 Ashmouni, 1 Acala plant. Temp. 41–42° C. Humidity 84–87 per cent. No infection.

Exp. 3. July 16th. 1 Hartsville, 1 Acala plant. Temp. 30–31° C. Humidity 87–93 per cent. One or two spots on a few leaves.

Exp. 4. July 19th. 1 Zagora, 1 American Upland plant. Temp. 24·8–25·8° C. Humidity 84–87 per cent. (but owing to the sticking of the hygrostat reached 95 per cent. for a short time). Heavy infection in two weeks.

Exp. 5. July 22nd. 3 Ashmouni plants. Temp. 24–25° C. Humidity 87–92 per cent. Fairly heavy infection in two weeks.

Exp. 6. Sept. 24th. 4 Ashmouni plants. Temp. 25° C. Humidity 83–86 per cent. Fairly heavy infection.

Exp. 7. Sept. 30th. 3 Ashmouni plants. Temp. 30° C. Humidity 83–86 per cent. Slight infection (2 leaves of one plant) after three weeks.

Exp. 8. Oct. 15th. 3 Ashmouni plants. Temp. 32° C. Humidity 83–86 per cent. Very slight infection on two plants after three weeks.

Exp. 9. Oct. 19th. 3 Ashmouni plants. Temp. 25° C. Humidity 69–71 per cent. Slight infection on two plants in three weeks.

Exp. 10. Oct. 25th. 3 Ashmouni plants. Temp. 27–28° C. Humidity 65 per cent. (but twice up to 70–74 per cent. for short time). Slight infection in three weeks.

Exp. 11. Nov. 2nd. 3 Ashmouni plants. Temp. 28° C. Humidity 64–66 per cent. No infection.

Exp. 12. Nov. 16th. 6 Ashmouni plants. Temp. 28–29° C. Humidity 58–61 per cent. No infection.

Since in each case at least two plants were used and each plant bore at least six leaves the surfaces of which were sprayed with an atomised suspension, it is clear that considerable replication was obtained, even though each separate experiment was not repeated. Ample opportunity for infection was provided since both sides of every leaf were thoroughly wetted with the suspension.

It is seen that both temperatures and humidity can apparently act as limiting factors.

Table I.

Exp. no.	Average temperature ° C.	Average relative humidity %	Infection
2	41	85	–
1	35	85	–
8	32	81	+
3	30·5	89	+ +
6	30	85	+ +
4	25	85	+ + + +
7	25	85	+ +
5	24·5	90	+ + +
9	25	70	+ +
10	27·5	65*	+
11	28	65	–
12	28·5	60	–

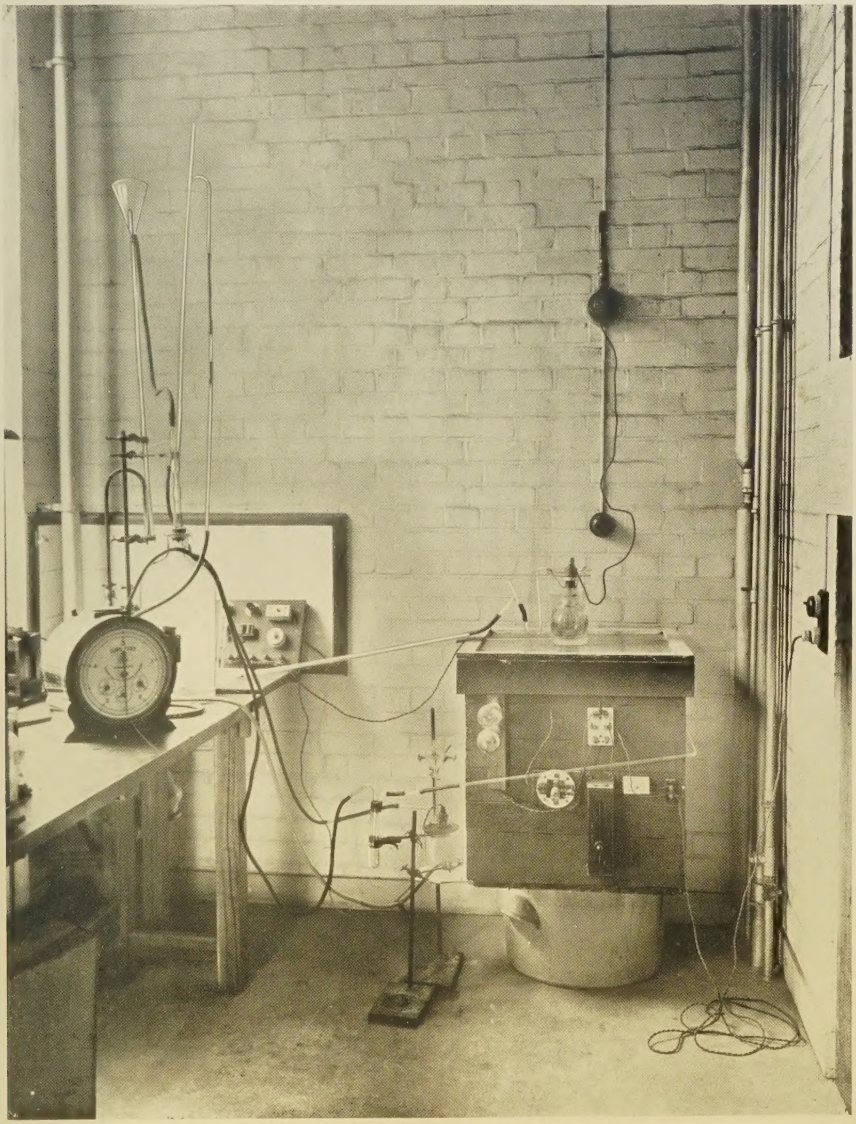
* Exp. in which humidity reached 70–74 per cent. for short time.

– = no infection; + = very slight infection; + + = slight infection; + + + = moderate infection; + + + + = heavy infection.

The results are shown in Table I, in which the experiments are arranged in two groups, the first with a high humidity (over 80 per cent.) and the second including those where the humidity was under 70 per cent. In the first group it is seen that above 32° C. no infection was obtained, while at that temperature very slight attack only was possible. Under such conditions of high relative humidity it appears that this temperature is the maximum at which infection can occur.

In the second group it is found that while slight infection was possible at 70 per cent. relative humidity, none was obtained at 65 per cent. humidity at a temperature of 28° C. (at which temperature infection occurs freely at high humidities).

Further experiments are being carried out to determine whether the limiting temperature varies with the degree of humidity and *vice versa*. It is possible, for example, that at the lower humidities infection may occur at lower temperatures such as 23–25° C. In the meantime it is clear that both factors are of considerable importance in the development of the disease. Temperature, of course, can hardly be controlled under field conditions, but cultural methods, such as good spacing of the plants, avoidance of overcrowding, even possibly the thinning of branches, which will help to prevent humid atmospheric conditions around the plant, may be of considerable importance in the control of the disease.



STOUGHTON.—On the Development of the Angular Leaf-Spot Disease of Cotton (pp. 333–341).

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Summary.

1. The serious disease of cotton caused by *Bacterium malvacearum* E. F. S. is associated with unfavourable climatic conditions.

2. A description of an apparatus for controlling air temperature and humidity within a chamber is given.

3. At humidities above 80 per cent. relative saturation the limiting temperature for attack by *B. malvacearum* was 32° C., above which infection did not occur.

4. At 70 per cent. relative humidity infection was slight at 25° C.

5. At lower humidities no infection was obtained at a temperature of 28° C.

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EXPLANATION OF PLATE XVIII.

Photograph of apparatus.

(*Received February 14th*, 1928.)

THE MOSAIC DISEASE OF THE HOP; GRAFTING EXPERIMENTS, I

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1. Infection of the Rootstock by the Scion.

In the investigations carried out at Wye College during the summer of 1926 Mr T. C. Thrupp grafted a number of hop plants growing in pots. In one set of experiments these plants (consisting of varieties known to be susceptible to mosaic disease) served as stocks which were grafted with scions obtained either from plants affected with mosaic disease or from the seedling variety M 45 which at the time was suspected of being a "carrier" of mosaic disease[1].

Thirty-four plants were grafted, 25 with mosaic-affected scions and 9 with scions of M 45. It was found extremely difficult to graft successfully when mosaic-affected scions were used, and although in some cases the scion remained alive for some time it eventually died without having made any growth. In several of the experiments where a scion of M 45 was used, the graft was successful and growth was made. Except in one case only, the 34 grafted plants showed no signs of mosaic disease in 1926. In the one exception, where a scion of M 45 was put on a plant of Tutsham, the leaves on 10 lateral branches which developed below the graft all showed mosaic-mottling. The details of this experiment (Exp. 11) are included with those given below.

In 1927, 13 of the 34 plants showed very pronounced symptoms of the mosaic disease. The appearance of these plants and the grafting process carried out by Mr Thrupp in 1926 are described in the following order:

Cases 1 *to* 6. Grafted in 1926 with a mosaic-affected scion.

Cases 7 *to* 13. Grafted in 1926 with a scion of M 45.

1. On May 28th, 1926, a pot-plant of Tutsham was grafted with a scion of Farnham Whitebine affected with mosaic and this was immediately grafted with a scion

[1] In other experiments carried out by Mr T. C. Thrupp, clone plants of M 45 served as stocks and by grafting on them healthy mosaic-susceptible scions, proof was obtained (1) that M 45 was in fact a carrier.

taken from the same Tutsham plant. In this way a length (8·8 cm.) of mosaic-infected bine was introduced into the graft. By July 5th a growth of 1 cm. was recorded but on July 13th the double graft wilted and died. There were then, in addition, three other healthy bines, 7 to 8 ft. high.

On May 18th, 1927, one shoot from this plant was observed to be strongly affected with mosaic. It was 44 cm. high and near the tip the internodes were short and growth had apparently ceased. All the leaves were mottled, those on the lower part of the bine being also much recurved. The tip of the bine and the upper leaves were brittle. There was one healthy shoot, 40 cm. high, but with the tip broken off. Three young shoots, 4·7 cm. long, were just starting growth.

2. On May 28th, 1926, a pot-plant of Tutsham was grafted with a scion of Eastwell Golding affected with mosaic, and on this was immediately grafted a further scion obtained from the same Tutsham plant. On June 8th the double graft was wilting and it soon died. On June 16th a further attempt to double graft was made, using a short length of mosaic-affected Eastwell Golding and on this a second scion of healthy Farnham Whitebine. The two scions together measured 20 cm. The double graft made no growth and soon died. On July 27th this plant had three other bines from 4 to 7 ft. high, and there were no signs of mosaic disease.

On May 18th, 1927, one bine of this plant, 66 cm. high, was clearly affected by mosaic disease. The leaves from the third to the ninth nodes (from the ground) were distinctly mottled. There were seven other shoots measuring from 29 to 120 cm. and two of these were very stiff near the tip—a feature suggestive of mosaic.

3. On May 28th, 1926, a pot-plant of Tutsham was grafted with a short piece of mosaic-affected bine (10·8 cm.) of Eastwell Golding, and on this was grafted a further scion from a healthy Tutsham plant. On June 8th the double graft was alive but no growth had been made and by June 17th the grafted scions were dead. On July 27th there were four other bines (2 ft., 5 ft., 7 ft., 7 ft.) and two short shoots both 1 ft. long and none of these was affected with mosaic disease.

On May 18th, 1927, this plant showed symptoms of disease. One shoot (28 cm.) was not climbing and the internodes were very short. At the six upper nodes the leaves were conspicuously mottled with yellow-green marks which were larger than usual. One shoot (28 cm.) had ceased growth and the tip of the bine was stiff and non-climbing. The leaves at four nodes from the top were thin in texture and there was slight but obscure mottling. One shoot (23 cm.) was very similar to the last but younger and with less pronounced symptoms. Another (19 cm.) resembled in appearance the first shoot mentioned.

4. On May 28th, 1926, a pot-plant of Tutsham was grafted with a double scion, the lower part of which was from a plant of Eastwell Golding affected with mosaic and the upper from the same Tutsham plant. On June 8th there were signs of wilting and the double graft died. When the plant was examined on July 27th there were two other bines, 6 and 7 ft. high, and both were healthy.

On May 24th, 1927, there were six shoots on this plant; one (64 cm.) showed unhealthy, possibly mosaic, symptoms at the tip and on the leaves at the second, third, and fourth nodes down. Two shoots (17·5 cm. and 7·5 cm.) were stunted and showed evident mosaic symptoms and three (38·5 cm., 17·5 cm. and 14·0 cm.) were healthy.

5. On May 15th, 1926, a pot-plant of Tutsham was grafted with a scion from a seedling variety GG 90. This scion was affected with mosaic, having four very incurved leaves above the place of grafting, and a curled stem. The leaves were of yellowish colour. There was one pair of leaves on the stock below the place of grafting. The scion died after a short time without making growth. On May 27th, 1926, a scion from a mosaic-affected plant of the variety Farnham Whitebine was grafted on and this also failed. No note was kept of the time during which the scions were alive in either of the above cases, but it probably did not exceed 14 days. On July 27th, 1926, this plant had three other bines (4 ft., 9 ft., 9 ft.) all of which were healthy in appearance.

On May 24th, 1927, there was one shoot (83 cm.) which failed to climb and fell away from the supporting stick; the uppermost leaves showed doubtful symptoms of the start of mottling. Four other shoots (17 cm. to 134 cm.) were healthy.

6. On May 28th, 1926, a pot-plant of Tutsham was grafted with a mosaic-affected scion of the variety Farnham Whitebine. This grew only 7 mm. and was dead after eleven days. On June 17th this shoot was cut off[1]. On July 27th, 1926, there were three other bines which were 6 to 8 ft. in height and healthy in appearance.

On June 1st, 1927, this plant had produced the following shoots. One bine of 107 cm. which was failing to climb at the tip, had the leaves at the twelfth to sixteenth nodes (inclusive) mottled with mosaic markings and the margins down-curved. Another shoot (20·5 cm.) was growing weakly; it had eight pairs of leaves and all the five upper pairs showed evident mosaic mottling. Three other shoots (7·5 cm., 9·0 cm., 24·0 cm.) were apparently healthy or showed only doubtful symptoms of mosaic disease.

7. On April 21st, 1926, a pot-plant of Eastwell Golding was grafted with a scion obtained from a clone plant (G 15) of the carrier M 45. At the time of grafting, the tip of the scion was 22 cm. from the soil level and by the end of May, 90 cm. On July 16th the scion was brown at the tip but was otherwise healthy. The length had increased to 151 cm. (approximately 5 ft.). No disease symptoms were seen on the two other bines on this plant, both of which were 8 ft. high.

On May 6th, 1927, all of the four shoots (44 cm., 49 cm., 140 cm. and 144 cm. in length) which had been produced were affected with mosaic, all showing mottled foliage to some extent.

8. On April 12th, 1926, a pot-plant of Eastwell Golding was grafted (1) with a scion obtained from the same pot, and (2) with a scion of M 45. Two days later a further control "self graft" was made. All three grafted bines and the only ungrafted bine showed no sign of disease during 1926, though an attack of "red spider" made accurate observation difficult after July. The scion of M 45 made no growth and was dead on May 14th, but the two self-grafted bines, and that which was ungrafted, reached a height of 8 ft.

On May 6th, 1927, this plant had produced six bines and all showed conspicuous mosaic symptoms; (1) 225 cm. long, with leaves mottled almost to the tip of the bine; (*b*) 80 cm. long, rather wiry and with all the leaves conspicuously mottled; (*c*) 126 cm. long, with the leaves mottled nearly to the tip of the bine; (*d*) 190 cm. long, with the leaves for three-quarters of that height distinctly mottled; (*e*) a blind shoot with

[1] No record was kept as to whether the cut was made at or below the place of grafting.

no terminal bud, 86 cm. long, and having all the leaves mottled; (*f*) 177 cm. long, with the leaves slightly mottled.

9. On April 12th, 1926, a pot-plant of Tutsham was grafted with a scion of M 45; this, however, died after eleven days. On the same day (April 12th) another bine was grafted with a scion obtained from the same Tutsham plant, and on April 26th this scion was again grafted with another similar scion. In this way a "self double graft" was attempted. It was not successful and was dead on May 6th. On May 11th another scion, obtained from a clone plant (Ref. No. 153) of M 45, was grafted on a bine of the Tutsham. This was recorded as alive on May 24th, but there were no further records until July 27th when it was noted to be dead. On June 17th a scion of M 45 was grafted on a bine of the Tutsham at 6 ft. from the ground. The tip of the scion died back and was cut off on July 2nd, when two lateral shoots were starting to grow from the scion. By July 27th this scion was dead. There was, on that date, one other ungrafted bine 8 ft. high. Neither on the two long bines nor on the short trailing "runners" near the soil were there any signs of mosaic disease.

On May 6th, 1927, this plant had three healthy shoots (66 cm., 43 cm. and 26 cm.) and two mosaic-affected shoots (46 cm. and 38 cm.) with all the leaves conspicuously mottled.

10. On April 22nd, 1926, a pot-plant of Tutsham was grafted with a scion of M 45; about 3 cm. growth was recorded up to May 7th. The graft, however, was not successful and the scion was dead three weeks later. On July 27th there were two other bines, 8 ft. high, which were quite healthy.

On May 24th, 1927, one shoot (173 cm.) showed the leaves at the eleventh to sixteenth nodes from the ground down-curled and mottled with mosaic markings. Two other shoots (14·5 cm. and 37 cm.) showed suspicious symptoms, and one (30 cm.) was healthy.

11. On April 21st, 1926, a pot-plant of Tutsham was grafted with a scion of M 45. They united satisfactorily and by July 27th the scion had grown to a height of 5 ft. and was strong and apparently healthy. On May 19th a second bine of this plant was grafted with a scion of M 45. To make this graft, a long bine of the stock plant (5 ft. high) was cut down close to soil level. This was also successful and by July 27th, the scion had reached a height of 5 ft. On June 17th a third bine of the Tutsham plant was grafted at 7 ft. from the ground with a scion of M 45 which was about 6 in. long. No growth of the scion took place but it remained alive up to July 27th, the date of the last record. By July 14th it was noticed that all leaves on the main bine below this graft were healthy but the laterals over a distance of five internodes (*i.e.* 10 laterals) all showed decided mosaic mottling of the leaves. The internodes through which the virus had presumably travelled were 22·5 cm., 27·5 cm., 24·0 cm., 20·0 cm. and 17·5 cm. long, and the 10 laterals measured from 1·0 cm. to 13·5 cm. There were no other bines on this plant.

On May 24th, 1927, mosaic disease was noticed. One bine (76·5 cm.) showed the leaves at the seventh, eighth and ninth nodes with doubtful mottling and the tip was still climbing normally. One shoot (31 cm.) showed all the leaves to within three nodes from the tip, conspicuously mosaic-mottled. Another (28 cm.) was not climbing normally at the tip but was falling away from the supporting stick. The leaves at the fifth to eighth nodes inclusive (from the base) were mottled. There were two more short shoots coming through the soil (5·5 cm. and 3·0 cm.).

12. On April 19th, 1926, a pot-plant of Tutsham was grafted with a scion of M 45, 7·5 cm. long. This was again grafted on April 29th with a healthy scion of Tutsham and the double graft was successful, though no very active growth followed. On July 5th it was suspected that mottling was becoming apparent on the new leaves of the terminal scion. On July 16th all young new leaves of the terminal scion showed slight mosaic mottling and slight distortion, while all the older leaves remained normal. On July 27th the total length of this double grafted bine was 15 in. There were two other bines, 4 ft. and 8 ft. long, which were healthy and in flower.

On May 24th, 1927, this plant had one shoot (44 cm.) with the leaves at the sixth to tenth nodes (inclusive) from the base conspicuously mottled. Another shoot (40 cm.) showed the leaves at the fifth to tenth nodes (inclusive) from the base also conspicuously mottled and the tip was beginning to fall away from the supporting stick. Another shoot (31 cm.) had the leaves at the seventh to tenth nodes evidently mosaic-mottled. A fourth shoot (13·5 cm.) had very short internodes; it was not elongating and was apparently "blind." All the leaves were mottled. There were also two short bines (16·5 cm. and 6·0 cm.) which were healthy.

13. On April 20th, 1926, a pot-plant of Tutsham was grafted with a scion of M 45. This scion measured 18·8 cm. when grafted, and it grew to a length of 68·0 cm. On July 27th there were two other bines, both of which were 9 ft. high and showed no symptoms of disease.

On May 24th, 1927, there were seven shoots on this plant, five of which (16 cm. to 26 cm. in length) showed no definite symptoms of disease and were apparently healthy. One shoot (49 cm.) bore leaves at the fourth, fifth and sixth nodes from the base affected with mosaic mottling and another (91·0 cm.) had a few leaves showing suspicious symptoms.

As will be seen from the details given above, the varieties used as stocks and which subsequently developed the disease, were Tutsham (11) and Eastwell Golding (2). The 21 plants which failed to show mosaic symptoms in 1927 were all of the Tutsham variety.

As regards the proportion of plants, in the two classes, which showed mosaic disease in 1927, the numbers are as follows: of 25 plants grafted in 1926 with mosaic-affected scions, 6, *i.e.* 24 per cent., showed the disease in 1927; of the 9 plants grafted in 1926 with scions of M 45, 7, *i.e.* 77·8 per cent., showed the disease in 1927.

It seems safe to conclude that the 13 plants, the cut bines of which in 1926 had been in contact with virus-carrying scions, were infected that year, the virus travelling through the (usually) short length of bine serving as the stock into the underground rootstock from which mosaic-diseased shoots were produced in 1927. The fact that a much higher percentage of plants grafted with scions of M 45 became affected than when mosaic-affected scions were used is probably not to be attributed to the greater potency of the virus in this "carrier" variety, but to the fact that in many cases the scion had united and had

made growth. If the infection had been due to some other cause than the grafting (such as *Aphis*), it would be difficult to explain why so high a percentage of the plants grafted with M 45 scions became affected as compared with the low percentage of plants grafted with mosaic scions.

Further, there existed a set of plants, kept in the same greenhouse and submitted to the same conditions during the whole of 1926 and 1927, which served as controls. These plants, which numbered 81, consisted of pot plants of the varieties Tutsham (73) and Eastwell Golding (8). Of the 73 plants of Tutsham, 11 were grafted in 1926 with healthy scions of this variety[1]; of the 8 plants of Eastwell Golding, 3 were similarly self-grafted[2]. The remaining plants of both varieties were left ungrafted. All these 81 plants remained free from the mosaic disease in 1927.

The fact that apparently in some cases, viz. where no growth took place and the scion soon died (see above, Exps. 2, 3, 4, 6), the mere contact of the cut tissues of a virus-carrying scion with the stock was sufficient to cause the infection of the latter, gives ground for the surmise that possibly infection may be produced by cutting the rootstock with a knife contaminated with the sap of a mosaic-affected plant or of a carrier.

It is hoped to carry out experiments to obtain evidence on this point which is of considerable practical importance, since in commercial hop growing the rootstock or "hill" of every hop plant is annually cut with a knife, the operation being continued from hill to hill and using the same knife throughout.

2. Resistance to Mosaic Disease shown by scions of the variety Fuggles.

Both in the Experimental Hop Garden at Wye and at East Malling Research Station, the variety Fuggles has for many years remained persistently immune from the mosaic disease. In the Trials of New and Commercial Varieties of Hops at East Malling, a number of commercial, as well as some of the new seedling, varieties have annually fallen victims to the mosaic disease, while in no case in the tests carried out from 1922 to 1927 has a single plant of Fuggles become attacked. Attention was first called to this fact in the *Report* for 1922(2) and here as well as in

[1] Of these, seven scions were obtained from the same plant which served as their stock and four from different plants of the same variety. Two of the eleven were double grafts. All the scions made a growth of 5 to 9 ft.

[2] Of these, two scions were obtained from the same plant which served as their stock and one from a different plant of the same variety. All the scions made a growth of 5 to 9 ft.

later *Reports* (3) details are given of the circumstances under which the resistance of Fuggles has been shown.

In 1927 it was decided to test the supposition that Fuggles was resistant—a supposition based only on strong circumstantial evidence—by the grafting (if possible) of scions of this variety on mosaic-affected bines. Early in April a "hill" of a certain seedling variety (Ref. no. OP 29)[1] in the Experimental Hop Garden at Wye was observed to be very badly attacked by the disease, only short mosaic-affected shoots having been produced. On April 29th, when the grafting was carried out, there were about a dozen such shoots up to nine inches high and having very brittle stems and petioles. In the process of grafting adopted, it was essential in order to secure rapid union that the diameter of the stock should be approximately equal to that of the scion and in the present case it was found that all the shoots, except two, on the crown of the diseased hill were too thick and therefore unsuitable to be grafted with the rather thin sappy shoots which were then the only scions of Fuggles available. The shoots chosen as scions were first pulled by hand from the rootstock of a plant of Fuggles (Ref. no. R 1/68 *a*) and were carried to the "hill" to be grafted. Here the operation in the case of both grafts was as follows: the mosaic-affected stem was cut transversely with a sharp scalpel at a height of about 2 inches above soil level. By making two cuts with a razor, extending 1·0 cm. downwards from the exposed surface and meeting in the centre of the stem, a wedge-shaped piece was removed. The base of the scion was next cut with the scalpel to a shape similar to that of the wedge removed from the stock and the scion was placed in position. Thin rubber tape was wound tightly round the stem to maintain close contact between the cut surfaces of stock and scion and the last turn of the rubber was made secure by means of a trace of rubber solution. The two grafted shoots were both covered with lamp glasses plugged with cotton wool and these were earthed up to three-quarters of their height to provide protection from the sun and a cool and moist atmosphere within the glass.

One of the scions died after 10 days but the other united satisfactorily

[1] This seedling was raised in 1919 by crossing a variety (Ref. no. B 20) of unknown origin with a male hop (Ref. no. J 34) raised from a German variety. It is not known whether the male plant is susceptible or not. The female parent (B 20) has proved both at Wye and at East Malling (2) to be extremely susceptible to the mosaic disease. The seedling OP 29 was planted out in the Hop Garden at Wye in November 1923, and proved to be a female plant; in 1924 the bines just reached to the top wire (11 ft. 9 in.); in 1925 and 1926 it was recorded as being "healthy and vigorous but not very fruitful," and the bines reached over the top wire.

and was allowed to grow out of the top of the lamp glass by the removal of the cotton wool. After about three weeks the lamp glass was taken away and the now rapidly-growing scion was trained up a string to the breast wire.

Further attempts were made to graft mosaic-affected shoots of OP 29 on May 21st, when two more scions of Fuggles from the same source as before were grafted, using the same method. One of these scions died and was removed on June 8th but the other grew successfully and was trained to the breast wire. At this stage, some of the lower leaves were removed from both of the growing scions and as they progressed, the stripping process was continued until, when the bines had nearly reached the top wire (11 ft. 9 in.) the leaves had all been removed to the height of the breast wire (4 ft. 8 in.)[1].

During this time (June to August) all the short mosaic-affected shoots from the rootstock had been dying away. A few fresh shoots appeared during June but these, like the others, soon turned brown at the tips and died, leaving on the hill only the two grafted bines which were strong and healthy and which finally grew over the top wire. Both bines flowered and produced cones with ripe seeds and at no time were any mosaic symptoms shown; moreover, there was no retarding effect on growth and the leaves were always of good colour and normal shape. It was observed that when the scion had reached a considerable height and was increasing in thickness, the stem (stock) below the graft failed to thicken appreciably. As a result, the wedge and callus of the scion far outgrew the shoulders of the stock and thus a sudden bulging of the bine was produced. The bine at the juncture of stock and scion proved however sufficiently strong, and sustained no injury from swaying and twisting caused by strong winds.

It is of interest to note that in the course of numerous attempts, both at Wye and at East Malling, to graft mosaic-affected stems, it has been observed that these, when cut across with the scalpel, are not so sappy as healthy stems and are somewhat hard and dry. This lack of moisture may contribute to the causes of the failure which so frequently results when grafting healthy scions on to mosaic stocks.

Six other healthy scions of the variety Fuggles were grafted on to three other mosaic-affected hills in the same hop garden at Wye on April 29th and May 21st but none of these was successful.

The above experiment shows clearly that the variety Fuggles is completely resistant to the virus of mosaic disease. It is certainly a

[1] This process, known as "stripping," is commonly adopted in hop growing.

remarkable fact that two scions put on a mosaic-affected plant should grow away at once, rapidly climb to over the top wire (11 ft. 9 in.), flower, and produce a crop of normal hops which ripened their seeds. From experience it can be stated with certainty that had the grafting not been done, the plant of OP 29 would not have survived that season, since the severity of attack was such that all its numerous bines soon died off before even reaching a foot in height. In the case of some "hills" attacked by mosaic disease, a few healthy bines may be produced among the diseased ones; in the present case the grafting was done on bines which were obviously severely affected with the disease and no healthy bines at all were produced. The vigorous nature of the growth may perhaps be accounted for by the fact that there were only the two grafted bines growing from the rootstock of a four-year-old plant, the usual number of bines trained up from a mature healthy hill being eight.

A very similar case to the above has been recorded(4). In Mr T. C. Thrupp's experiments, scions of the "carrier" seedling M 45 were grafted on to mosaic stocks. The scions grew vigorously; we may quote here Mr Thrupp's description of the results obtained. "In view of its known history, it was not expected that the two scions of M 45 would develop the mosaic symptoms. It was, however, surprising to find that one of these throve to such an extent that it grew to a height of about 4·5 m. and remained free from all symptoms of the disease, while the remaining bines of the mosaic stock were nearly all dead from the severity of the attack."

The variety Fuggles having proved in our experiment completely resistant to the disease, the question naturally arises as to whether this variety may be a "carrier" like M 45. It may be mentioned here that certain experiments (the details of which will be published later) carried out in 1927 by Mr D. Mackenzie and the present writers, give some evidence that this is the case.

Summary.

1. Certain hop plants, which were grafted in 1926 with scions either (*a*) of plants affected with the mosaic disease, or (*b*) of the seedling variety M 45 (previously proved to be a "carrier"), produced in 1927 shoots affected with mosaic disease.

2. From the evidence available, it would appear that the virus of mosaic disease can travel down a grafted stem and infect the rootstock.

3. It would appear, further, that mere contact of a virus-carrying scion with the stock (with no growth of the former taking place) is sufficient to cause the infection of the rootstock.

4. When scions of the commercial variety Fuggles were grafted on a hop plant severely affected with the mosaic disease they remained healthy and completed their full seasonal development.

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INOCULATION EXPERIMENTS WITH THE DOWNY MILDEWS OF THE HOP AND NETTLE (*PSEUDOPERONOSPORA HUMULI* (MIY. ET TAKA.) WILS. AND *P. URTICAE* (LIB.) SALMON ET WARE)

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When describing in this *Journal* in 1925(7) the appearance and rapid spread in England of a Downy Mildew of the Hop, we put forward hypotheses to account for various phenomena observed. The views were expressed (1) that the Downy Mildew of the Nettle (*Pseudoperonospora Urticae*) and that of the Hop (*P. Humuli*) might prove to be one and the same species; and (2) that the Downy Mildew of the Hop was endemic in England. In the light of evidence which has since come to hand, the whole problem of the sudden appearance in this country of a new parasitic fungus on the Hop bears a new aspect. From the information published since 1925 by Baudys(1), Blattny(2), Flachs(3), Riols(4), and Vermeulen(9) it may be surmised that in common with those of Great Britain, the wild and cultivated hops of the Continent of Europe began to be generally attacked perhaps round about the year 1920 by *P. Humuli*, a species new to Europe and till then known only in Japan and in one State (Wisconsin) of North America. The evidence shows that by 1923, or earlier, this fungus was present on Hops in Germany, Moravia and Yugoslavia.

Historically considered, then, the advent of *P. Humuli* into Europe and its subsequent rapid spread afford a parallel case to that of the Vine Downy Mildew (*Plasmopara viticola*). It is clear to us, therefore, that in 1924(6) we were mistaken in supposing that *P. Humuli* was endemic to this country.

As to the manner of introduction of the fungus into Europe, only speculations exist. Apart from its occurrence in the Experimental Hop Garden at Wye College in 1920, the fungus was not found in hop gardens in England until 1924; in several countries on the Continent, however, it appears that commercial gardens were invaded, as noted above, in 1923

or earlier. It is of course possible that the occurrence at Wye in 1920 was connected with an earlier importation of hop plants or seeds from Japan. It seems clear, however, that the outbreaks on the Continent are unconnected with the above, for the fungus could hardly have spread from England between 1920 and 1923 and have infected hops in Germany, Yugoslavia, and other countries on the Continent (where, it is now known, outbreaks occurred in gardens in 1923 or perhaps earlier) without having invaded during those years the hop gardens of the south of England. Hence, if spores of this fungus can be transported in the air to considerable distances, it would appear (apart from any question of the prevailing winds from March to September) more likely that the hop gardens in the south-east of England became infected in 1924 by spores from the Continent than that the contrary movement took place. With regard to the first outbreaks on the Continent, no information has been published connecting them with any importation of hop plants from outside Europe. Dr Blattny has put forward ((2) p. 150) the theory that possibly the fungus may have spread by natural means from Asia to Europe, viâ Yugoslavia.

Fresh evidence has been obtained, from experiments described below, that *P. Humuli* is able to attack to some extent species of other genera than *Humulus*, viz. *Urtica* and *Parietaria*. These facts, if they indicate an extension of the host range of *P. Humuli* in Europe, have an economic significance which is discussed later.

Experimental.

The methods employed in all the experiments were as follows. Spores were collected by means of a moist sterilised camelhair brush and transferred to a watch-glass containing distilled water. It was found that after a period varying from 50 minutes to several hours, the zoospores emerged[1] and when a large number were seen to be in active motion, the brush was used to place drops of the water containing zoospores on the under surface of leaves to be inoculated. It was always ascertained that zoospores were still swimming actively in the last remaining drops of the water. The opposite leaf (at the same node) to that inoculated provided a control and a drop of distilled water was placed, using a

[1] Zoospores usually frequent the upper surface of a drop of water, or water in a watch-glass, whereas the full and also the empty sporangia mostly sink to the bottom. The zoospores, when they come to rest, do so at the surface of the liquid and near to the edge of the water. When, however, zoospores are placed in a large hanging drop on a cover-slip, they move about just under the cover-slip and not nearer to the air at the bottom of the hanging drop.

fresh brush, on its lower surface. In the following experiments, the special control leaf remained healthy unless a statement is made to the contrary. The number of uninoculated leaves on a plant is occasionally detailed to give some idea of the quantity of healthy foliage.

Inoculated plants were usually kept covered with a bell-jar or inverted beaker for 48 hours and after that period were exposed to the air of the laboratory for 1 to 12 hours and were then again covered to ensure moist conditions overnight. The alternate covering and uncovering was continued until the end of each experiment.

Seedling plants grown in pots were used. Their leaves were young and tender and were thought to be in the best condition to become infected. Some of the seedling hop plants used in the later experiments during October were, however, not in a condition of vigorous growth.

Sections which were cut in order to investigate the extent of penetration or internal spread of the mycelium were mounted in an alkaline solution of azo blue, using the method described in a previous article (10).

1926.

Exp. 1. On May 25th affected shoots of a "wild" hop from a hedge at Crouch near Wrotham, Kent, were brought to the laboratory and from these a zoospore suspension was made. Three seedling plants of *Urtica urens* were inoculated. After three days a dark spot, showing on both surfaces, appeared on one of the two inoculated leaves of the second plant. The leaf was removed and two conidiophores bearing spores were seen with the microscope on the upper surface and two younger conidiophores with no spores, near by. On the lower surface, five straight unbranched hyphae, which were evidently young conidiophores, were projecting from the centre of the dark area. This leaf was placed in a damp Petri dish and further conidiophores developed only on the dark area. On June 8th, 14 days after the inoculation, three small dark spots with four or five conidiophores with spores were found on both the inoculated leaves of plant 3. The dark spots were all situated at the recesses of the serrated margin of the leaf.

No infection occurred on the three inoculated leaves of plant 1.

Exp. 2. On May 25th four seedlings of *U. urens* were heavily inoculated on their growing tips with the same suspension of zoospores as was used in Exp. 1. The suspension was placed on about four of the leaves round the apical bud of each plant. None of the plants became infected on any of their leaves within 14 days, after which they were thrown away. The plants used were from the same stock as in Exp. 1.

Exp. 3. On May 28th the following were inoculated with material from the same source as in Exps. 1 and 2; (*a*) three plants of *U. urens* growing singly in separate pots; (*b*) three hop seedlings each in a separate pot, and growing with each, one plant of *U. urens* in the same pot.

With respect to (*a*), the plants were inoculated on 3, 4, and 1 leaf respectively. Only the last became infected and showed on the sixth day one small spot with three conidiophores. With respect to (*b*), the first pot containing the two plants was

examined on June 3rd, when one of the two hop leaves inoculated was found infected. Two days later the other hop leaf also showed conidiophores. On the nettle in the same pot, two leaves and one cotyledon had been inoculated but none became infected. In the second pot, the one inoculated hop leaf showed a small group of conidiophores on the thirteenth day (June 10th); the inoculated cotyledon and leaf of *U. urens* remained uninfected. The hop plant in the third pot was inoculated on two leaves and both of these became infected in small spots on the thirteenth day. Two leaves and one cotyledon of the nettle in the same pot had been inoculated and on the thirteenth day, both leaves, but not the cotyledon, showed small spots with conidiophores and spores.

1927.

Exp. 1. On May 5th a zoospore suspension was obtained from affected cultivated hop plants growing in the Experimental Hop Garden at Wye. Two leaves on each of nine, and one leaf on each of three, seedling plants of *U. dioica* were inoculated. Two leaves on each of six hop seedlings (about 8 in. high) were also inoculated. On the sixth day after inoculation, seven of the twenty-one nettle leaves showed dark areas and shrivelling of the epidermis but no conidiophores were found. There was no such effect on the opposite control leaf at each node, nor on any of the other leaves. On the eighth day (May 13th) the plants were discarded.

On the sixth day, seven hop leaves were found to be heavily infected and with numerous conidiophores on the lower surface. On the eighth day, ten leaves were infected; the remaining two were both on one plant and this was kept until the twelfth day (May 17th) when one of the leaves showed brown discoloration; only two conidiophores, however, were seen. No infection resulted on the other leaf.

Exp. 2. The inoculum used[1] was from the same source as in Exp. 1. On May 6th two leaves on each of six plants of *U. urens* were inoculated, one leaf on each of six hop plants, and of the Japanese Hop (*Humulus japonicus*), one cotyledon and one leaf on each of four plants and two leaves on each of two plants.

On the third day (May 9th) slight effects were seen on all the inoculated plants. On that day it was only possible to examine minutely one plant of *U. urens*. One leaf, which was removed, had a dark area 4 mm. × 8 mm. confined by veins and showing through on both surfaces; this was thickly covered on the lower surface with conidiophores which were seen protruding from the stomata. Sections of the leaf showed mycelium more in the spongy parenchyma than between the palisade cells, though here and there hyphae had reached the upper epidermis. On the other inoculated leaf there was a large dark brown dried patch, both above and below, measuring 4 mm. × 9 mm. and bounded by veins. On this there were no conidiophores visible with a lens. This leaf was cut off and was kept in a damp Petri dish for two days, when about twenty scattered conidiophores were seen with the microscope.

On May 11th all the plants were examined:

(1) *U. urens*. The second plant showed on one leaf a large blackish brown area on which were dense masses of conidiophores with spores. This leaf was cut off and the fungus was used for preparation of the inoculum for Exp. 3. The other leaf of this plant showed a large dark area; no conidiophores were visible with a lens and it was not until May 16th (ten days after inoculation) that a very few scattered

[1] The conidia produced zoospores within 50 minutes—the shortest time for this process that we have observed.

conidiophores were found. The third and fourth plants were each infected on both their inoculated leaves. These showed dark-coloured areas with conidiophores and spores and conspicuous puckering of the leaf margin. All four of these leaves were removed to provide the inoculum for Exp. 3.

The fifth plant showed one leaf densely covered with conidiophores on the lower surface of a rather withered and perished area. This leaf was much puckered and curled downwards and was removed for the purpose mentioned above. The other leaf showed no effect of the inoculation but conidiophores were found on this on May 16th. At no date were dark-coloured areas formed. The sixth plant had, on one leaf, two dark patches running in a line along one vein. Conidiophores were dense on one of these and fairly numerous on the other and along the vein itself. This leaf was also removed. The other leaf was discoloured and infected below; downy masses of conidiophores and spores were present on the patches between the veins.

The plants were about 6 in. high and had five pairs of leaves expanded, in addition to those on lateral shoots which arose from most of the lower axils. All these other leaves, as well as the controls, remained healthy throughout the experiment.

(2) *H. Lupulus.* On each of the six seedling plants the lower surface of the one inoculated leaf became covered with black masses of conidiophores and spores. The discoloration and puckering of the leaf were first observed on the third day (May 9th) and the presence of conidiophores was confirmed on the fifth day (May 11th). In addition to the opposite control leaf, six other leaves and the cotyledons on each plant remained healthy.

(3) *H. japonicus.* The six seedling plants were examined on May 11th. Of the four cotyledons inoculated, on four separate plants, three showed no effect but one was turned slightly brown at the place where the inoculation drop had hung. On each of these four plants, one foliage leaf had been inoculated; one showed no effect, two were marked with a very slight pale discoloration, and one with a large brown area. The remaining two plants had been inoculated each on two leaves. The first had a small brown mark on one leaf and on the other there was only slight puckering; the second plant showed large angular brown spots on one leaf and slight puckering of the other. Conidiophores were not present on any of the above leaves. On May 13th there was no alteration in their condition. On May 16th—ten days from the start of the experiment—two or three solitary conidiophores with spores were found on the one leaf described as having a large brown area on May 11th. No further infection of any leaves, whether inoculated or not, took place and the plants were discarded on May 16th.

Exp. 3. The inoculum used was obtained from leaves of *U. urens* infected with spores taken from the hop (Exp. 2). These leaves had been cut from the nettle plants on May 11th and had been kept overnight to produce more spores. On May 12th, at 10.35 a.m., the spores were brushed into water in a watch-glass. Both brush and watch-glass had been kept in alcohol for 5 min. previously. The day was dull and cold (laboratory temperature 14·5° C.) and the germination did not start till 1 p.m., *i.e.* after 2½ hours. Zoospores were moving in fair numbers at 1.45 p.m. when the drops of inoculum were placed on the under surface of the one marked leaf of each of ten hop seedlings. On the fifth day after inoculation (May 17th) the ten hop plants were examined and in every case conidiophores and spores were found. On the inoculated leaf of each of the first three plants there was a single group of conidio-

phores with spores, causing slight puckering of the leaf. On the fourth plant, four groups of densely crowded conidiophores with spores occurred on the inoculated leaf and a general infestation was starting all over its lower surface. The leaf on the sixth bore a densely crowded mass of conidiophores with spores on one small area measuring 6 mm. × 2·5 mm., the inoculated leaves on the fifth, seventh, ninth and tenth plants showed no result, and on the eighth plant there was one small dense group of conidiophores.

All the plants on which conidiophores had been found (six in number) were destroyed and the remaining four were kept for further observation. Two days later (May 19th) the fungus appeared on the inoculated leaf of each of these; on three of the leaves there was only one small but dense group of conidiophores covering an area of 3 mm. diameter, and on one, the lower surface was almost completely covered with conidiophores and spores. The upper surface of this last leaf was of a pale yellow-green colour immediately above the areas most infected.

Exp. 4. The inoculum was obtained from various affected hop plants in the Experimental Hop Garden at Wye. Diseased shoots were brought to the laboratory on May 13th and were kept under a bell-jar until the following day. The weather was dull and cold on May 14th and the laboratory temperature did not exceed 14° C. The spores were a mixture of young and old and did not readily produce zoospores. They were placed in water at 11.45 a.m. and the first few zoospores were seen at 1.30 p.m., *i.e.* after $1\frac{3}{4}$ hours. The suspension was left until 7 p.m. ($5\frac{1}{2}$ hours after the first movement had been seen) and the water was then found to be full of actively swimming zoospores. The inoculations were made between 7 p.m. and 7.30 p.m. Some of the suspension remained over, and it was observed that zoospores were actively swimming until 11 p.m. that night, but judging from the number that were rounding off preparatory to production of a germ tube, this must have been nearly the end of the period of activity.

Six each of the following were inoculated, on one leaf only of every plant: *U. urens*, *U. dioica*, *H. Lupulus* and *H. japonicus*. Three additional plants of *U. dioica* were then inoculated (two leaves on two plants and one leaf on the other) and three additional plants of *U. urens* (two leaves on each).

The first examination was made on May 19th (five days after inoculation):

(1) *U. urens*. Four plants were infected and two showed no result. On plant 1 there were numerous but not densely clustered conidiophores. The infected leaf showed a dark, rather water-soaked area (1 cm. diam.) visible on both surfaces when in a moist atmosphere, but light grey in colour when in dry air. On plant 2 there were only a few scattered conidiophores and a faint grey discoloration (about 0·75 cm. diam.). Plant 3 showed no result, except that the lower epidermis was less shiny in well-defined areas. On plant 4 there were numerous conidiophores thinly effused over the lower surface. There was no noticeable discoloration. Plant 5 showed no effect of inoculation. On plant 6, conidiophores were scattered thinly over one area. There was no marked discoloration though the whole area was faintly grey and the lower surface was dull as opposed to the glossy green of the rest of the leaf.

On May 23rd, plants 3 and 5 were again examined. The former showed a few scattered conidiophores with spores and the latter, one large area thinly covered with conidiophores and one very small blackened area with densely clustered conidiophores. Thus within nine days all the plants of *U. urens* first inoculated had become infected,

while the control leaf and all the rest of the leaves (numbering eleven in each case) on every plant remained healthy.

All the three additional plants (six leaves) showed no result on May 19th. On May 23rd the first showed puckering of both leaves, but no conidiophores; the second bore about six conidiophores close to the mid-rib of one leaf and showed no result on the other leaf; the third was exactly similar to the second plant. Thus two leaves, of the six inoculated, became weakly infected.

(2) *U. dioica*. The six strongly growing seedlings had each four pairs of leaves expanded. They showed no results of the inoculation either on May 19th or on May 23rd, and the plants were then thrown away. The three additional plants (five leaves) similarly gave negative results.

(3) *H. Lupulus*. The six plants were examined on the fifth day (May 19th) with the following results: (1) and (6) no effects of the inoculation were seen; (2) the leaf showed two areas, of slightly pale yellow colour, distinguishable on both surfaces, with densely crowded conidiophores; (3) conidiophores were fairly dense all over the lower surface between the main veins where there was a somewhat pale yellowish colour; (4) and (5) on one area only there was a fairly dense mass of conidiophores and no discoloration of the leaf.

On May 23rd, when the plants were next examined, (1), (5) and (6) were black with conidiophores and spores on the under surface of the inoculated leaf which showed a yellowish discoloration above; (2), (3) and (4) were also black on the under surface of the inoculated leaf, and brown angular spots were beginning to become well defined on the upper surface. There were six or eight leaves on each of the young plants and they, including the control leaf, were in all cases unaffected.

(4) *H. japonicus*. The young plants had three pairs of leaves expanded. They showed no result of inoculation either on May 19th or on May 23rd when they were thrown away.

Reviewing the positive results obtained in this experiment, with respect to *U. urens* it could not be said on the ninth day (May 23rd) that the fungus had become firmly established as it had in the case of the six hop plants similarly kept. On the nettle, the upper surface of the leaf was not much darkened by May 23rd, but the infected leaves were decidedly puckered and the margins were down-curled. All the other leaves on the six plants were flat and normal; there were as many as 36 on each, including those on lateral branches. Spores were obtained from the infected nettle—and hop—leaves, and a comparison was made of their size. In the case of the fungus on *U. urens*, only granular, obviously ungerminated spores were measured in order to avoid any possibility of including spores actually placed on the nettle leaf with the suspension of zoospores nine days previously. Very few conidia were obtainable, the process of infection and establishment of the fungus having apparently come to a standstill. On the hop plants, innumerable conidia could be obtained from the inoculated leaves and it was to be expected therefore that the range of variation of the conidia from the hop would be far greater than in the case of those from the nettle. Twenty from both host plants were measured. Those from *U. urens* averaged $22\mu \times 13\mu$ (18μ–$28\mu \times 12\mu$–16μ) and those from the hop averaged $23\mu \times 17\mu$ (20μ–$26\mu \times 14\mu$–18μ). The mode of the spore measurements from *U. urens* was $20\mu \times 12\mu$ or 14μ and from *H. Lupulus* $24\mu \times 16\mu$.

Exp. 5. Basal spiked growths of cultivated hops were collected at Crouch, Kent,

on May 21st and brought to Wye on May 23rd. They were kept under a bell-jar in the laboratory until the following day, when a suspension of zoospores was obtained. The room temperature was warmer than formerly (18·5° C.) and during the progress of the experiment it varied between 14·5° C. and 19° C. Six plants (in pots) of *U. urens* were inoculated on two leaves each, and six plants of *H. Lupulus* also on two leaves each. Twelve seedling plants of *U. urens*, which had been pricked out and were growing in the open, were inoculated each on one leaf only. The plants were then covered with lamp glasses plugged with cotton wool. Hot sunshine on May 26th caused saturation of the atmosphere inside the lamp glasses and the plugs were removed and were not replaced.

(1) *U. urens*. The first examination of the pot plants was made three days later (May 27th) when eleven leaves of the twelve showed a grey coloration, often on a well-defined area, on the lower surface. These areas varied in size and sometimes consisted only of one of the projections of the serrated margin. On the seventh day (May 31st) the final examination of the plants was made and on seven of the twelve inoculated leaves, conidiophores were found. Only on one plant were both the leaves found infected. On each of the other five plants one leaf gave a positive result. Conidiophores, when present, were in some cases thinly scattered over most of the lower surface of the leaf. This was greyish below, though not discoloured above, and was often puckered. In other cases the conidiophores were confined to smaller grey coloured areas and here again there was no discoloration of the upper surface.

(2) *H. Lupulus*. The six plants were examined on May 27th (three days after inoculation) when five of the twelve leaves showed symptoms of infection in the form of areas, pale grey on the lower surface, and greyish green on the upper. Occasionally the epidermis above the greyish patches was peculiarly shining. The plants were first able to be examined again on June 4th (eleven days after inoculation). On plant 1 the entire tip of the upper leaf was puckered and there were withered greyish areas between the veins. A few conidiophores were seen by use of the microscope. The lower leaf was turned a brownish green colour from the apex to the base and only along the central part of the lamina. No conidiophores could be found but a suspicion that some internal spread of the fungus had taken place was strengthened by the fact that the dark area extended towards the petiole. Sections of the leaf were cut and the mycelium was traced down the full length of the petiole as far as the junction with the main stem. Here it was lost and was not found either in the stem or in the rootstock.

Both leaves of the second plant showed angular brown patches, greyer on the lower surface; some of these were definitely killed. A few conidiophores were found on both leaves, only by means of the microscope.

The upper leaf of the third plant showed areas which were killed and which bore a dense mass of conidiophores on their lower surface. The leaf was distorted and there were patches on the upper surface which were slightly sunk and brown in colour. On the lower leaf there were several angular brown patches with densely grouped conidiophores; there was slight puckering, and some areas were killed through to the upper surface.

The fourth plant showed several grey angular spots on the upper leaf; these were quite dry, noticeably thin, and brown coloured on the upper surface. There were no conidiophores. The lower leaf was in exactly the same condition.

The lower surface of the upper leaf of the fifth plant was of a very pale green colour from the tip of the lamina to the place of junction with the petiole. There were no conidiophores, but internal spread of the fungus was assumed to be taking place. The lower leaf had grey-green dead areas which were brown above. No conidiophores were present.

No effects of inoculation were visible on the lower surface of the upper leaf of the sixth plant, but on the upper surface a large, pale yellow-green area extended from the apex of the lamina to the petiole, but did not reach the margins. It was suspected that the fungus had entered the petiole and the hyphae were in fact traced, by means of longitudinal sections, as far as the main stem. No conidiophores were found. The under surface of the lower leaf was dry and grey over a large area from the apex to the petiole but not reaching the margins. This area was brown on the upper surface. There were no conidiophores and hyphae were not found in the petiole.

The plants of *U. urens* out of doors were examined on May 31st. The one leaf inoculated on eleven of the twelve plants could be distinguished without close inspection, owing to the presence of one or more grey-black areas on the upper surface. Conidiophores were found on the eleven leaves, but in no case were they as dense as they commonly are on leaves of the hop. They were, indeed, so scarce on six of the leaves that it was necessary, in order to confirm their presence, to pick the leaves and bring them to the laboratory for microscopic examination. On four of these there were only from four to ten conidiophores. On the remaining seven, conidiophores were fairly densely grouped on the lower surface of the dark-coloured areas, but they were never more than about half as dense as in the maximum infection of hop leaves. Some of the conidiophores were of large size, with over twenty spores on each. On the one leaf which bore no conidiophores, there was one large dark area and two smaller; all of these were grey on the lower surface.

Exp. 6. On June 23rd a "spiked" hop bine received from Tenbury, Worcestershire, on the previous day, was used for the supply of zoospores. The following plants were inoculated that evening. *H. Lupulus*, six plants, six leaves; *Parietaria ramiflora*[1], six plants, twelve leaves; *U. dioica*, eight plants, ten leaves.

The water in which the zoospores were moving was left overnight and considerable numbers being still in movement on the following morning (June 24th), the same liquid was used for inoculating *H. japonicus*, six plants, six leaves; and *U. urens*, six plants, twelve leaves. None of the plants was covered, but the leaves were kept frequently moistened by atomising.

(1) *H. Lupulus.* The six seedling plants were examined on July 1st (eight days after inoculation), and on two of them a brown mark was evident on the inoculated leaf. On removal of these leaves, it was found that there was internal mycelium but no conidiophores. Three of the remaining leaves on July 4th showed light-coloured marks or streaks and in every case hyphae were found in the mesophyll. Conidiophores were not present. One of the six leaves inoculated showed no result.

(2) *P. ramiflora.* Frequent examination was made with a lens but no conidiophores were seen on the inoculated leaves. On July 4th and following days the leaves were examined and then sectioned. In nine, the whole upper surface was covered with minute brown flecks which had been apparent since June 29th. The lower

[1] *Parietaria ramiflora* Moench = *P. officinalis* L. pro parte.

surface was similarly flecked, but this was partly hidden by the covering of hairs. Hyphae were found in the mesophyll and running between the palisade cells. One haustorium was clearly seen in a large cell of the upper epidermis. Groups of dead or brown cells were found in the mesophyll, and a hypha was usually found in proximity. In three, the very minute spots were present in smaller numbers, and hyphae and dead cells were again found.

(3) *U. urens.* Ten of the twelve leaves of the six plants showed one or more brown areas and were puckered but no conidiophores were present. On the remaining two leaves there were no effects of inoculation.

(4) *H. japonicus.* The one inoculated leaf on each of the five plants showed no results and on that of the sixth plant there was one pale brown dead area measuring 9 mm. × 6 mm.; no conidiophores were found.

(5) *U. dioica.* Nine of the ten leaves of the eight plants gave no result, but on the remaining one there were nine angular brown spots measuring from 1 mm. to 5 mm. × 2 mm.

Exp. 7. A "spiked" lateral shoot from a cultivated hop plant (Ref. no. R 4/82 *a*) in the College Hop Garden was brought in on June 30th and kept in the laboratory until July 2. The zoospores obtained were numerous and active and the inoculum appeared therefore specially satisfactory. The plants inoculated were not kept covered, but all their leaves were atomised with water at intervals. The inoculations were made on *H. Lupulus*, six plants, six leaves; *P. ramiflora*, five plants, fifteen leaves; *H. japonicus*, ten plants, ten leaves; *U. urens*, six plants, eight leaves; *U. dioica*, eight plants, eight leaves.

On the fourth day (July 6th) one of the hop leaves (*H. Lupulus*) was marked with decidedly pale areas of lighter green colour. On *U. urens*, the leaves inoculated were slightly puckered; there were no effects visible on any of the leaves of *Parietaria*, and on *U. dioica* one leaf alone showed a pale area on the upper surface. No general production of conidiophores developed, so the plants were kept an unusually long time and were examined finally on the seventeenth day (July 19th).

(1) *H. Lupulus.* Only one leaf of the six showed a few scattered conidiophores. It had also four large pale areas with internal mycelium extending nearly to the petiole. These measured 1·5 cm. × 0·5 cm. Another leaf had three pale areas in which hyphae were found. The remaining four leaves showed no effect of inoculation.

(2) *U. urens.* From one to three grey-brown areas were seen on seven of the leaves and no effect on the eighth. In no case were conidiophores found.

(3) *U. dioica.* No infection occurred on any of the eight leaves.

(4) *H. japonicus.* None of the ten leaves showed any effect of inoculation.

(5) *P. ramiflora.* The first ten leaves examined showed no external signs of infection, except a slightly paler green area on the upper surface. A small quantity of mycelium was found in the lamina in every case, the hyphae being swollen into lobes and not spreading far from the probable place of infection. The majority of hyphae were in the mesophyll and were extended towards the palisade cells. The next two leaves showed a similar pale area, which however was covered on the lower surface with small brown spots. In each case mycelium was found. Two leaves showed small areas which were brown on both surfaces. Traces of mycelium were found but the hyphae were not spreading. One leaf, also with internal hyphae, was marked with a paler green colour on the lower surface only.

Exp. 8. Leaves of *U. dioica* attacked by *Pseudoperonospora Urticae*, were collected at Wye on September 23rd and were kept in a moist atmosphere in the laboratory. In spite of the low temperature at night, conidiophores developed on the brown angular patches which, when the leaves were collected, showed little trace of the presence of the fungus. On September 25th, a suspension of zoospores was obtained after three hours; other spores, notably of *Macrosporium* and of *Phoma*, were also present in the water. Inoculation of one leaf on each of the following plants was commenced at 2.15 p.m.: *H. Lupulus*, thirteen small seedlings; *U. urens*, ten seedlings; *U. dioica*, ten seedlings.

On September 26th, the room temperature was raised to 18°–20° C. All the plants were kept covered to preserve a moist atmosphere, except during a few hours each day. On September 28th, the first darkening of the leaf was seen on two plants of *U. dioica* and on two of *U. urens*. Further examination of the plants gave the following results:

(1) *U. dioica*. The ten seedlings were all 3 or 4 in. high and had from three to five pairs of leaves expanded. The inoculated leaf was in all cases young and recently unfolded.

All the plants became fully infected. On the fourth day the majority of the inoculated leaves were puckered, and on the fifth day conidiophores were found on all. The upper surface showed a yellow-green or dark coloration.

(2) *U. urens*. The plants used for inoculation were young and had from five to six pairs of leaves expanded. In every case the last node but one from the tip was chosen and one leaf was inoculated. The height of the plants was between $3\frac{1}{2}$ and 5 in. All the leaves were examined on the seventh day after inoculation (October 1st) and the results were as follows: Plants (1) to (3), the upper surface was grey-black in large angular patches running along the veins. Dense and grey coloured masses of conidiophores occurred on the lower surface of the patches. (4) The lamina was much puckered in addition to being marked with patches as described above. Numerous conidiophores were present. (5) to (10) The lamina was puckered and a dark area of about 1 sq. cm. occurred on the upper surface. Conidiophores were plentifully scattered over the lower surface.

(3) *H. Lupulus*. On October 1st, none of the plants showed any result except a very slight yellowing and translucency of the inoculated leaf. On October 3rd the plants were examined and the leaves sectioned to discover whether any penetration had taken place. The seedlings measured from $1\frac{1}{2}$ in. to 4 in. in height and had from three to five pairs of leaves expanded. The inoculation was always on one of the younger leaves. Plant (1) showed a faint yellow-green translucent appearance on the upper and lower surfaces, but no conidiophores. Hyphae were found in the mesophyll and extending between the palisade cells as far as the upper epidermis. (2) There was slight shrivelling or withering of the lamina, but no discoloration. Hyphae were not found internally. (3) There was a very dark brown dead area (7 mm. × 5 mm.), visible on both surfaces of the lamina. No conidiophores were found. Hyphae extended from the mesophyll to the upper epidermis and were in considerable quantity in the part of the lamina which was turned brown. (4) A yellow-green area (7 mm. × 5 mm.) occurred on both sides of the lamina. Hyphae were plentiful from the mesophyll to the upper epidermis and in one case two short outgrowths, possibly young or rudimentary conidiophores, were seen projecting from a stoma. (5) Blotches

of yellow-green colour occurred on both surfaces of the puckered lamina. Six scattered conidiophores were found on the lower surface. There was no doubt that they were growing from the leaf and had not been introduced with the inoculum, their firm attachment being shown by the fact that they were seen to twist around in the normal way in currents of air when they were viewed under the microscope. Internally, hyphae were found extending throughout the coloured zones and were particularly abundant in the mesophyll. They grew and branched amongst the palisade cells. Small, typically nodular, haustoria were seen in cells of the palisade tissue and in the upper epidermis. One section showed a group of four oogonia, with a maximum diameter of 40μ, situated in the palisade tissue. In another section there was an oogonium (34μ diam.) at the top of the palisade cells and lying immediately beneath the upper epidermis. Conidiophores having been found, it was decided to leave the remaining plants for a further ten days. Nos. (6) to (13) were examined on October 13th and the results were as follows: (6) A brown area (5 mm. × 5 mm.) contained mycelium but bore no conidiophores. (7) There were two brown marks similar in all respects to (6). (8) Two brown areas (5 mm. × 4 mm.) occurred, with only a single conidiophore. Hyphae were found in the lamina. On (9), (10) and (11) there were no effects of inoculation. (12) The lamina was slightly crinkled. A lighter area (3 mm. × 4 mm.) showed up plainly on the dark green upper surface, and on this the epidermis was not so glossy as elsewhere. Hyphae were plentiful in the mesophyll. There were no conidiophores. (13) The lamina was much puckered and three brown areas occurred (each 3 mm. diam.) surrounded by a lighter zone. Hyphae were found internally and six small conidiophores were seen, each with only four spores.

Exp. 9. On October 3rd, leaves of *U. dioica* attacked by *P. Urticae* were collected at Wye from which, on October 5th, a suspension of zoospores was obtained. The following plants were inoculated: *U. dioica*, four plants, four leaves; *H. Lupulus*, seventeen plants, seventeen leaves; *U. urens*, four plants, sixteen leaves. All the plants were kept in two large glass tanks near a window in the laboratory. A small quantity of water was placed on the floor of the tanks in order to make the atmosphere sufficiently moist. One side of the tank was removed for at least an hour each day to provide fresh air. To secure similar conditions for all the plants, which were too numerous to place in one tank, the hops and nettles were divided as evenly as possible between the two. The temperature remained fairly constant at 20° C.

On the fourth day (October 9th) the fungus was already established on *U. dioica* and examination of all the plants was immediately made. (1) *U. dioica*: The four seedlings were from 5 to 7½ in. high and either five or six pairs of leaves were expanded. One leaf of the fourth pair from the ground was inoculated in each case. Plant (1) Two groups of small black spots were visible on the upper surface of the lamina. Each group measured 5 mm. × 4 mm. The lower surface was grey-black, with a dense mass of conidiophores and spores extending further than the sharply defined spots on the upper surface. There were nine healthy control leaves and twelve additional leaves on small laterals which were also healthy. (2) One grey-black area (4 mm. × 3 mm.) was present on the upper surface of the lamina. On the lower surface, however, the fungus was found over an area measuring 18 mm. × 11 mm. Here the conidiophores were dense and formed a grey patch which was bounded by the larger veins on each side of the mid-rib. There were nine other control leaves and sixteen additional

leaves on small laterals which were all healthy. (3) The lamina was slightly puckered. There was no black spotting but only a yellowish green coloration on the upper surface. Conidiophores were dense on the lower surface over an area 30 mm. × 17 mm. which was bounded as described above in (2). There were nine other control leaves and eleven additional small leaves on lateral growths, all of which were healthy. (4) Faint grey spots were beginning to appear on the upper surface. On the lower, conidiophores were dense in a grey mass which measured 20 mm. × 12 mm. The area on which they were situated was also bounded as in (2). There were eleven other leaves and in addition twelve small leaves on lateral growths, all of which were healthy.

(2) *U. urens.* The four plants were also examined on the fourth day (October 9th). They were from 6½ in. to 8 in. high, and had from seven to nine pairs of leaves expanded. Each plant had five pairs of leafy laterals from 1½ in. to 3¼ in. long, and in some cases the young leaves on such laterals were selected for inoculation in preference to the larger leaves on the main stem.

Fourteen of the sixteen leaves inoculated were infected. On the lower surface of three, the conidiophores were densely grouped and on the remainder they were thinly scattered. The majority of the fourteen leaves were puckered and showed a slight alteration of colour on the upper surface.

On these four plants, 267 leaves were counted and only the fourteen inoculated leaves were infected.

(3) *H. Lupulus.* The plants used for inoculation were seedlings which were not vigorous nor growing strongly. On October 9th, none of the seventeen leaves showed any result and the plants were kept until October 15th. Some of the uninoculated leaves on three plants became infected by that date; three inoculated leaves bore a few conidiophores on the lower surface, and fourteen gave no result.

Owing to the fact that ten days had elapsed since the inoculation and that uninoculated leaves had become infected, reliance could not be placed on the three positive results obtained. The spores from one of these inoculated leaves (on a plant with healthy controls) were washed off into a watch-glass and a suspension of zoospores obtained with which plants of *U. dioica* were inoculated (see Exp. 10).

Exp. 10. The suspension of zoospores used[1] came from a hop plant which had been inoculated with *P. Urticae* taken from *U. dioica* (see Exp. 9). Two leaves on each of two plants of *U. dioica* were inoculated on October 15th, but by the end of ten days (October 25th) no infection was observed. The negative result obtained gives, therefore, no corroborative evidence that the fungus observed on the three hop seedlings in Exp. 9 was really the result of infection by *P. Urticae.*

Exp. 11. On October 21st, leaves of *U. dioica* attacked by *P. Urticae* were collected from a single plant growing in a hop garden at Wye and using a zoospore suspension from these, the following plants were inoculated on October 22nd: *U. dioica*, two plants, two leaves; *U. urens*, two plants, two leaves; *H. Lupulus*, five plants, five leaves.

All of these were kept in one large glass tank. They were examined on October 25th, *i.e.* after the completion of three days. Both the plants of *U. dioica* showed, on

[1] The zoospore suspension was not plentiful and no young hop seedlings were then available for inoculation.

the inoculated leaves, dense violet-grey tufts of conidiophores around the inoculation drop which had not yet completely dried up. These two plants were removed from the tank and were kept separately until October 28th when the two leaves had become grey with conidiophores on the lower surface. There were twenty-five other leaves on each plant and all of these were healthy.

The two plants of *U. urens* on October 25th showed spots of darker green on the inoculated leaves but no conidiophores were seen. On October 28th, both of the leaves showed scattered conidiophores on the lower surface. On each plant there were eleven other leaves and all these were healthy.

The five hop plants which were somewhat stunted seedlings and making no vigorous growth, showed no effect of the inoculation and were destroyed on October 28th.

Exp. 12. Leaves of *U. dioica* attacked by *P. Urticae*, were collected at Wye, and, using a suspension of zoospores, the following plants were inoculated on October 22nd: *U. dioica*, six plants, six leaves; *H. Lupulus*, ten plants, ten leaves; and *H. Lupulus*, four plants, four cotyledons. The plants were kept under bell-jars or inverted beakers to preserve a moist atmosphere.

No infection was observed on any of the plants on October 25th. They were next examined on November 1st, with the following results:

1. *Urtica dioica*. (1) Conidiophores scattered in groups on the lower surface; not densely crowded. The remaining 21 leaves on the plant were healthy. (2), (3) and (4) Conidiophores numerous on the lower surface. Brown spots (about 1 mm. diam.) visible on upper surface. The remaining twenty-nine leaves on plant (2) and twenty-seven on both of plants (3) and (4) were healthy. (5) Only four minute brown spots (1 mm. diam.) with conidiophores below each. The remaining twenty-one leaves were healthy. (6) Numerous brown spots on upper surface and conidiophores present on the lower. The remaining twenty-five leaves were healthy.

2. *H. Lupulus*. There was no discoloration and no sign of the fungus on any of the ten leaves inoculated. As in Exp. 11, the plants were not in vigorous growth. Three of the four cotyledons of *H. Lupulus* which had been inoculated showed no effects and no hyphae were found internally. In the fourth, however, there was a greyish green area (4 mm. diam.) on the lower surface. Two isolated conidiophores, with spores, were seen but it could not be determined with certainty whether they were actually growing from the tissues of the cotyledon. Sections showed a general invasion by hyphae and typical nodular haustoria in the upper epidermal cells. Although opposite control cotyledons were in all cases healthy, the above must perhaps be considered as a doubtful case of infection.

Discussion.

The experiments described above show that, under certain conditions, plants of *U. urens* can be infected by *P. Humuli*. If we consider apart the few experiments made in 1926, we find that the percentage of successful infections obtained when spores were taken from the Hop and sown on *U. urens* was 15·4. In 1927, in those experiments concerning *U. urens*, spores of *P. Humuli* when sown on 36 hop leaves gave 18

infections, or 50 per cent., and when sown on 68 leaves of *U. urens* gave 38 infections, or 55·9 per cent. In all the 38 cases of infection, conidia were produced. The percentage here of successful infections on *U. urens* is considerably higher than that obtained by us in 1924, viz. 13·3 per cent. In one case (Exp. 3) recorded above, spores produced on *U. urens* as the result of infection by spores taken from the hop, were sown on 10 hop leaves and full infection was obtained on each leaf. The evidence may therefore be regarded as conclusive that *P. Humuli* can attack and form spores on *U. urens*. It is obvious that here we have a fact of possibly economic significance, since the question of controlling the disease on cultivated hops is directly affected if there are other species of plants on which, under natural conditions, the fungus can exist and form spores. *U. urens* is not uncommon as a weed among, or near, cultivated hops, at any rate in the south of England; whether it can actually assist in spreading the disease is dependent on whether *P. Humuli* can, in nature, not only temporarily infect this plant but also establish itself there. As regards this last point there is as yet no evidence; we have not observed secondary infections on plants of *U. urens* such as would indicate that the fungus from the hop is able to thrive.

With respect to the other species used in our experiments, the results were either negative or inoculation was followed only by the production of mycelium which remained barren. In the case of *U. dioica*, 50 leaves were inoculated, with entirely negative results as regards the production of conidiophores and spores. In the same experiments, 30 leaves of the hop were inoculated and 18 became infected; *i.e.* 60 per cent. In Exp. 4, all of the 6 hop leaves inoculated became infected, while no signs of infection resulted on the 11 inoculated leaves of *U. dioica*. In Exp. 1, where 11 of the 12 hop leaves inoculated became infected, 7 of the 21 leaves of *U. dioica* showed dark areas and shrivelling of the epidermis probably resulting from zoospores having attacked the leaf cells. In Exp. 6, one leaf of *U. dioica*, of the 10 inoculated, showed brown angular spots indicative of attack.

The results obtained are somewhat different from those which we have previously recorded with *U. dioica* in 1925(7), when 5 leaves out of 27 leaves inoculated, or 18·5 per cent., became infected and produced conidiophores.

With regard to *H. japonicus*[1], on one plant only (Exp. 2) was inoculation followed by sub-infection, *i.e.* by the formation of a large

[1] The plants of *H. japonicus* were raised from seeds supplied by Messrs Vilmorin-Andrieux and Co. and by Messrs Ryder and Son, Ltd.

brown area of leaf tissue on which a very few conidiophores with spores were produced. A few of the other inoculated leaves showed a discoloration indicating probably some penetration by the fungus. In Exps. 2 and 4, the 12 hop leaves all became fully infected (100 per cent. infection); of the 18 leaves of *H. japonicus* inoculated in the same series, only one (as noted above) showed sub-infection. In Exp. 6, 6 leaves of *H. japonicus* were inoculated and only one (on which one pale brown, dead area occurred) showed any signs of having been attacked; in this experiment, however, none of the 6 hop leaves inoculated became fully infected, although in 5 the presence of mycelium was observed. The evidence obtained in Exps. 2 and 4, where 100 per cent. infection was obtained on the hop, would seem to indicate clearly that *P. Humuli* was unable to attack *H. japonicus*. The fact is interesting because other investigators have recorded that *H. japonicus* can be infected. According to Prof. Miyabe it occurs on this host in Japan, and more recently Dr Blattny (2) (pp. 43 and 172) has recorded that in June, 1926, he found in Bohemia plants of *H. japonicus* attacked rather severely[1] by *P. Humuli* and that he infected *H. japonicus* with spores taken from a "wild hop[2]."

The different results obtained here with *H. japonicus* may possibly indicate the existence of physiologically distinct strains of *P. Humuli*. It may be noted that Dr Blattny (2) has brought forward some evidence for this, which however can hardly be considered as conclusive. The question whether specialisation of parasitism exists in *P. Humuli* is one of practical importance, since it may be intimately connected with the resistance shown by certain commercial varieties of hops in this and other countries (2, 8).

With regard to the inoculations on *Parietaria* (Exps. 6 and 7), mycelial invasion with the formation of a varying amount of brown flecking of the upper and lower epidermis was consistently found in all the 27 leaves inoculated. This was the more noteworthy since in both these experiments none of the 12 hop leaves inoculated became fully infected, one leaf showing only a few scattered conidiophores, and only 6 of the other 11 leaves showing internal mycelium. It remains to be seen whether under certain conditions *P. Humuli* is able to form spores on *Parietaria*.

It may perhaps prove to be the case that *P. Humuli*, having now found its way into Europe, will extend gradually its range of host plants. When in nature its spores are carried to species of genera related

[1] "...ein ziemlich starker Befall."
[2] "...vom südböhmischen verwilderten Rothopfen."

to *Humulus*, a process of adaptation to the new host may be taking place. In this connection it is certainly interesting to find that artificial inoculations have shown that it can infect *Urtica urens*, and attack to some extent *U. dioica* and *Parietaria ramiflora*.

Considering now the question of the infection powers of *P. Urticae* towards the hop, positive results were obtained in one experiment only (Exp. 8); here 13 hop leaves were inoculated with spores from *U. dioica* and 3 showed "sub-infection," with the production of mycelium and 1 to 6 conidiophores. Of the remaining 10 leaves, 6 showed the presence of brown areas and internal mycelium. In the same experiment 10 leaves of both *U. dioica* and *U. urens*[1] were inoculated and all became fully infected. In one experiment (Exp. 11), spores from *U. dioica* infected fully *U. urens*, but no signs of infection occurred on the 5 inoculated hop leaves[2]. In Exp. 12, the 6 inoculated leaves of *U. dioica* became infected, but of the 14 hop leaves inoculated only one (a cotyledon) was perhaps infected. In our previous work (7) some evidence was obtained that spores from *P. Urticae* on *U. dioica* could cause sub-infection on the hop.

It may be noted here that investigators on the Continent, *e.g.* Blattny (2), have obtained only negative results when endeavouring to transfer *P. Urticae* to the hop and *P. Humuli* to *Urtica*.

Summary.

1. The evidence now available indicates that the Downy Mildew of the hop, *Pseudoperonospora Humuli* (Miyabe and Tak.) Wils. is a distinct species coming from Japan, or possibly North America, into Europe about 1920. The views advanced by us in 1924 that this species might be co-specific with *P. Urticae* and that it is endemic to England, are abandoned.

2. Inoculation experiments have shown that *P. Humuli* is capable of infecting and producing spores on *Urtica urens*. No evidence was obtained, however, as to whether it can establish itself permanently on this host-plant.

3. Spores produced on *U. urens* as the result of inoculation with *P. humuli* can infect fully the hop.

4. Zoospores of *P. Humuli* placed on leaves of *Urtica dioica* and *Parietaria ramiflora* produced phenomena approaching sub-infection.

[1] Dr Blattny has recorded (2) similar results, without however giving details of the experiments.

[2] The hop plants used were not growing vigorously owing to the late season (October 22nd).

Table I.

Exp. No.	Source of inoculum	Plant inoculated	Date	No. of leaves	Result No. of leaves Pos.	Neg.	Remarks
1	*H. Lupulus* (wild) Crouch	*U. urens*	25. v. 26	7	3	4	
2	*H. Lupulus* (wild) Crouch	*U. urens*	25. v. 26	16	0	16	
3	*H. Lupulus* (wild) Crouch	*U. urens*	28. v. 26	16	3	13	
		H. Lupulus		5	5	0	
1	*H. Lupulus* (cultd.) Wye	*U. dioica*	5. v. 27	21	0	21	7 show some evidence of penetration
		H. Lupulus		12	11	1	
2	*H. Lupulus* (cultd.) Wye	*U. urens*	6. v. 27	12	12	0	
		H. Lupulus		6	6	0	
		H. japonicus		12	1	11	External effects visible on some
3	*U. urens**	*H. Lupulus*	12. v. 27	10	10	0	
4	*H. Lupulus* (cultd.) Wye	*U. urens*	14. v. 27	12	8	4	
		U. dioica		11	0	11	
		H. Lupulus		6	6	0	
		H. japonicus		6	0	6	
5	*H. Lupulus* (cultd.) Crouch	*U. urens*	24. v. 27	12	7	5	
		H. Lupulus		12	5	7	7 show penetration
		U. urens†		12	11	1	
6	*H. Lupulus* (cultd.) Tenbury	*H. Lupulus*	23. vi. 27	6	0	6	Internal mycelium in 5
		P. ramiflora		12	0	12	Internal mycelium in all
		U. dioica		10	0	10	Brown angular spots on 1
		H. japonicus		6	0	6	Brown angular spots on 1
		U. urens		12	0	12	Brown areas on 10
7	*H. Lupulus* (cultd.) Wye	*H. Lupulus*	2. vii. 27	6	1	5	Internal mycelium in 1
		P. ramiflora		15	0	15	Internal mycelium in all
		H. japonicus		10	0	10	
		U. urens		8	0	8	Brown areas on 7
		U. dioica		8	0	8	
8	*U. dioica* Wye	*H. Lupulus*	25. ix. 27	13	3	10	Internal mycelium in 6
		U. dioica		10	10	0	
		U. urens		10	10	0	
9	*U. dioica* Wye	*U. dioica*	5. x. 27	4	4	0	
		U. urens		16	14	2	
		H. Lupulus		17	3	14	Probably 3 accidental infections
10	*H. Lupulus*‡ (cultd.) seedling)	*U. dioica*	15. x. 27	4	0	4	
11	*U. dioica* Wye	*U. dioica*	22. x. 27	2	2	0	
		U. urens		2	2	0	
		H. Lupulus		5	0	5	
12	*U. dioica* Wye	*U. dioica*	22. x. 27	6	6	0	
		H. Lupulus		14	0	14	Hyphae in 1 of 4 cotyledons inoculated

* The spores on *U. urens* which were used, were produced after inoculation from the hop in Exp. 2.

† *U. urens* growing out-of-doors.

‡ The spores on *H. Lupulus* which were used, were produced after inoculation from *U. dioica* in Exp. 9.

5. Since *P. Humuli* in its native countries has been observed only on species of *Humulus*, the fact that in Europe a species of *Urtica* can be artificially infected, may indicate a widening of its host range.

6. Spores of *P. Humuli*, taken from cultivated hops, failed to infect fully *Humulus japonicus*.

7. Spores of *P. Urticae* (Lib.) Salmon et Ware, taken from *Urtica dioica*, were found to infect fully *Urtica urens*.

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ON *DEMATIUM PULLULANS* De B. AND ITS ASCIGEROUS STAGE

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(With Plates XIX and XX, and 5 Text-figures.)

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Introduction.

The fungus *Dematium pullulans* was first described and named by de Bary in 1866, and since that time its relationship to other fungi has been a frequent subject of controversy. The object of the present paper is to give an account of this organism such as will enable its position in the scheme of classification to be assessed.

De Bary (1) described the fungus[1] as consisting of a septate mycelium from the segments of which ellipsoidal cells sprout freely at the ends or the sides. After exhausting the available food supplies the hyphae divide transversely into cells which are as long as broad; these swell up into roundish shaped segments with two-layered walls and contents rich in oil, meanwhile assuming a brown colour. These resting cells ("gemmae"), on renewal of activity under favourable conditions, again yield ellipsoidal sprout cells, either directly or from a branched mycelium which develops first. The free sprout cells also bud copiously, and closely

[1] This fungus does not belong to the genus Dematium (Pers.) Sacc. *Syll. Fung.* IV, 308, and Rabenhorst's *Krypt. Flora*, VIII, 688.

resemble Saccharomyces; or, under adverse conditions they may undergo change to gemmae. De Bary stated that *D. pullulans* was probably nearly related to Fumago or Pleospora. A common form is illustrated in Text-fig. 1.

This simple description has been modified by other investigators, who have considered the fungus to be a more complicated organism. Löew (10) pointed out the similarity between the resting mycelium and uniseptate gemmae of *D. pullulans* and analogous structures of *Cladosporium herbarum* Lk.; but because these fungi developed differently when transferred to fresh media he concluded that there was no genetic relationship between them. Laurent (9), believing he observed aerial conidiophores of Cladosporium arising from the mycelium of Dematium in culture, and that he had produced in cultures derived from the same source Cladosporium in darkness and Dematium in sunlight, stated that *C. herbarum* and *D. pullulans* were different forms of one fungus. This view was accepted and given in works published subsequently by Frank, Constantin, and Saccardo (14). Massee (11) stated that the gemmae of Cladosporium yielded *D. pullulans* on germination, as did also fragments of conidiophores when placed in water. There is much evidence that Laurent's view is erroneous, and modern mycologists do not now accept it. Janczewski (7) and Planchon (12) repeated Laurent's experiments but did not get similar results, and Planchon contended that the original results were the outcome of a mixed culture. Massee's work, as pointed out by Hoggan (6), is open to similar criticism. The errors in both cases were, doubtless, due to those similarities noted by Löew (*l.c.*), and to the fact that these two fungi are so frequently associated in nature. Brooks and Hansford (5), whilst investigating *C. herbarum*, observed no indication of its connection with *D. pullulans*, and the writer has grown and maintained in pure culture under many and diverse conditions for some five years different strains of both these fungi, and under no circumstances has one reproduced the other. De Bary's suggestion of a possible connection between *D. pullulans* and Fumago was disposed of by Zopf (17), who showed that the gemmae of the latter when placed in fresh media yielded mycelia bearing conidiophores or pycnidia.

That *D. pullulans* was a stage in the life-cycle of some Ascomycete has been suggested many times. The earlier observers, from erroneous interpretation of certain morphological phenomena as being endogenous spore formation, concluded that the organism was one of the yeasts or allied fungi, but the correct interpretation, given by Klöcker and

Schiönning(8), disposed of this view. Most other suggestions were based on a similarity between *D. pullulans* and the conidial stage of some Ascomycete. Brefeld(4) pointed out its resemblance to the conidial stages of *Plowrightia* (*Dothidea*) *ribesia*, *P.* (*D.*) *puccinioïdes*, and *Sphaerulina intermixta*, eventually stating that *D. pullulans* was the conidial stage of the last named. This was proved to be incorrect by Klöcker and Schiönning (*l.c.*), who showed that there was an unmistakable difference between the two fungi. Genetic relationship between *D. pullulans* and *P. ribesia* was disproved by Hoggan(6), as the two fungi differed essentially in parallel cultures. Whilst *D. pullulans* could not be connected with any definite Ascomycete, there remained the theory of Berlese(3), mentioned also by Brefeld(4), that this was a collective name for the conidial stages of several Ascomycetes. This theory has special significance in connection with the present contribution, which will show that though there are different forms of the conidial stage, these are not phases of different Ascomycetes, but strains of *D. pullulans*, and that this has its own ascigerous stage.

Forms Isolated: Cultural Characters.

D. pullulans, of common occurrence on moribund and dead vegetable matter, a notably frequent companion of *Cladosporium herbarum*, and a follower of various pathogenic fungi, has been isolated by the present author from more than a dozen different kinds of host plants. Many of these isolations yielded an exactly similar form of the organism; but some forms, even from a single source, showed slight morphological and definite cultural differences. The forms selected for detailed comparison here are some which show a gradual transition of characters, so that the appearances presented by the extreme members are very different from one another. The forms and their origins are as follows:

WI, WII—from wheat leaf, associated there with *C. herbarum*.

CI, CII—from dead cherry flower associated with *Monilia cinerea*.

AsI—from the ascigerous stage occurring naturally on wheat.

AsII—from wheat after inoculation with AsI.

When infected material is held in a moist chamber at about 18° C., *D. pullulans* grows freely, as a rule, appearing as a very delicate, white mycelium, which soon becomes of a greenish tinge, somewhat like *C. herbarum*. The mycelium yields bud-spores ("sprout cells") abundantly, and these were used for obtaining cultures of all the strains mentioned above except AsI. Strain AsI was raised from ascospores (see p. 381), obtained from perithecia formed under natural conditions. Isolations

were made from each strain by the following method, taking all possible precautions to ensure aseptic conditions at each stage of the process. Spores were transferred on the point of a platinum needle to drops of water on cover-glasses, and from suitable drops transfers were made with a platinum loop to a series of drops; of these, the hanging drops which, under the microscope, showed only a few perfectly separated spores, with total absence of any other fungus spores, were used for dilution cultures in malt gelatine and malt agar in Petri dishes. Of the numerous dishes poured for any one strain, three were selected which showed well isolated colonies. From each of six (when there were so many) colonies on each of these dishes transfers were made in duplicate to four kinds of media, and their subsequent growth compared. The similarity and maintenance of growth characteristics on each kind of medium for any one strain was taken as evidence that cultures of that strain had been secured from a single conidium or single ascospore respectively[1].

The cultural characters are detailed for a malt gelatine medium only, the characters on other media being given briefly and in a comparative manner. Special attention is suggested to Plates XIX and XX, which supplement this description. Simultaneous culture on a number of media is essential, since strains which appear very similar on one medium may show distinction in macroscopic appearance on another; the media here mentioned serve this purpose for the strains under consideration, and also serve to show the gradual transition of characters from strain to strain. The growth described below as moist and colourless consists of scanty, hyaline hyphae with innumerable bud-spores ("sprout cells"), the whole becoming pasty and of a buff shade on ageing; the olive or olive-black coloration depends upon the formation of chlamydospores ("gemmae") from either bud-spores or mycelium, or both, and develops in characteristic manner.

On Malt Gelatine. (Plate XIX, fig. 1.)

Strain	Week	
AsI	1	Streak first a delicate, white mycelium, then slimy and pale buff, with laterals[2] transversely wrinkled and covered with a silvery bloom with some green.

[1] These first-poured plates were always kept for several weeks; foreign growth, except bacteria and occasionally Penicillium, was exceedingly rare on them, and cultures from contaminated plates were never used as stock for sub-cultures.

[2] For conciseness, "streak" is used for the line of inoculation; "margin" for the edge of the actual streak; "laterals" for the medium between the margins and the edges of the slant.

Strain	Week	
AsI	2	Streak slimy, laterals wrinkled crusts, olive-green to olive-black in parts, bearing silvery bloom.
	4	Streak soft, rest of surface deep olive-black, contorted crust, which sinks to the glass as medium liquefies.
AsII	1	Streak very moist and spreading, covering slightly wrinkled laterals with a cream then very pale buff, wet growth, which drains into the angle.
	2	Entirely liquefied, only a thin brownish film with a few olive flecks remaining adhering to the glass above.
WI	1	Streak moist, then somewhat waxy; laterals puckered.
	2	Whole surface moist and pale buff; spongy elevation at base.
	4	Entirely liquefied, leaving on the glass above a thin, warm buff to brown residue.
WII	1	Streak moist, extending to moist or pasty, very pale buff laterals.
	2	Whole surface pale buff, irregularly wrinkled, spongy at base.
	4	Whole surface moist, deep buff, finely wrinkled and parts spongy; later olive to olive-black at the top and edges; little or no liquefaction.
CI	1	Streak first a delicate, white mycelium, then moist and tinged buff, with laterals transversely puckered.
	2	Streak as before, laterals deeply wrinkled, brown with olive-green along the crests, the whole covered with white to silvery aerial bloom.
	4	Surface wrinkled, spongy and moist below; snuff- to dark-brown throughout; medium slightly liquefied. Later growth remains on glass as a wrinkled layer with a little scattered olive-black.
CII	1	Streak creamy, sunken, slightly spongy below; laterals delicately wrinkled transversely, moist surface.
	2	Much liquefaction, leaving pale buff, pasty layer above, and little spongy growth with traces of olive at the base.
	4	Surface a wet, buff paste above, somewhat spongy with a little olive below. Later sinks to orange-brown, marginal residue with little olive black.

Strain AsI is of outstanding character, the surface growth being a contorted crust, deep olive-black in colour, sinking to the glass as the medium slowly liquefies. Strains WII and CI show gradations away from this, with less olive-black growth and more creamy growth, and rather more rapid liquefaction of medium. WI and CII with little or no olive-black growth, much creamy growth from the first, and rapid liquefaction of the medium, lead into the type AsII with creamy, buff growth only and very rapid liquefaction of the medium.

On Malt Agar (Plate XIX, fig. 2). AsI shows some corrugation and olive-black coloration of the medium within one week after inoculation, and eventually a much corrugated black crust, with a few slimy residual patches. AsII, at the other extreme gives a smooth, waxy surface of buff colour, with traces only of olive at the bottom of the streak, and the outermost edges of the slant. Between these extremes the transitions are WI, CI, CII, and WII successively, passing from somewhat crusted, olive-black surface to smooth, waxy, buff coloured surface growth.

On Wheatmeal Agar (Plate XIX, fig. 3). The forms fall into two groups as on malt agar, namely AsI, WI, CI, marked by obvious olive growth, and AsII, WII, CII

without distinct olive growth. This applies to two-month old cultures, Fig. 3 showing the appearance at one month.

On Hard Potato-Dextrose Agar (Plate XX, fig. 4). These cultures furnish an example of reversal of amount of coloured growth on strongly acid medium (*p*H 2·5 to 3·0) due to added citric acid; the dark growth is submerged mycelium in the laterals of the slant. The transitions are:

AsII, with olive-black laterals and well-marked deep olive superficial growth thereon.

CII, almost as AsII, but sparse aerial olive growth on the laterals.

WII, WI, and CI, intermediate stages leading into AsI.

AsI, with very slow production of olive in the laterals.

The maintenance of the transitional gradations in this reverse direction is remarkable evidence of the constancy of the series.

On Salts-Dextrose Agar (Plate XX, fig. 5). On this medium the most distinctive features occur in the streak itself and its margins. AsI shows a buff, pasty linear streak with well-developed olive-black, raised, "oily" margins, the whole becoming eventually a tough, black layer. WI is very similar, but the streak blackens rapidly and the margins are cream to warm buff. The transition towards increase and permanency of buffy streak with diminution and loss of margins is through CI, CII, to WII, and AsII. This medium, and the corresponding saccharose medium, induce a gradual, though very slow modification of strain characters after repeated transfers. AsI and WI, for example, increase the pinkish buff growth of the streak and reduce the continuous "oily" margins to a series of greenish black, marginal blobs, not always continuous, so approaching the AsII type. But the strains are not reduced to a common type after several years of culture.

Influence of Hydrogen-ion Concentration. The characteristics on the salts-dextrose agar, and salts-saccharose agar, at *p*H 6·6, mentioned previously, were compared with those on the same media at *p*H 5·5 and *p*H 7·8. The increase of hydrogen-ion concentration showed some slight influence on the submerged mycelial growth in the laterals and the development of the olive, or olive-black, stages; these phases were slightly favoured for CI, CII, AsII, inhibited for WI, and unchanged for AsI and WII. Decrease of hydrogen-ion concentration consistently favoured the yeast-like phase, and repressed more or less completely the mycelial growth and formation of the resting phases from both bud-spores and mycelium. Neither acidity nor alkalinity of media induced more prominent differences between the strains, or retarded the general growth of the organism.

Influence of Protein. Media of different kinds, but all containing the same amount of protein and peptone (2 per cent. each), were adjusted to *p*H 7·0, and the strains grown thereon at room temperature. Protein agar gave a very slow growth as a thin surface film, becoming waxy as it extended and eventually reaching the edges of the slant; protein alone, apparently, provided the elements necessary for scanty growth. Protein-potato agar gave only slightly better growth, whilst protein-salts agar gave more abundant and rapid growth, showing that the organism was favoured by a good supply of mineral matter. None of the strains yielded any resting (olive) phase by the end of three months. By comparing the growth on salts agar, with the carbonaceous matter supplied as protein and as dextrose (p. 376) respectively, both

at *p*H 6·6, it was evident that this protein was the factor inducing bud-spore development and repressing mycelial and chlamydospore phases. Somewhat similar results were noted on materials differing in amount of vegetable protein. Wheat grain with crude protein content of 12·1 per cent. gave a mere sprinkling of black (chlamydospore stage) amongst a considerable amount of white (bud-spore) growth. Polished rice grain with a crude protein content of 6·7 per cent., on the contrary, gave moderately rapid and eventually abundant black growth.

From the foregoing accounts it will be observed that the best method of distinguishing between the strains is by growth on media containing "simple" natural nutrients, at a hydrogen-ion concentration of about 6·6. Variation of hydrogen-ion concentration of sugar and potato media within the limits stated, and addition of protein, were of no assistance in distinguishing more clearly between the strains. A second object of these cultural studies was the production of the ascigerous stage *in vitro*, but this was not favoured by increase of acidity, alkalinity, or protein content. Immature perithecia, as described later, were produced on substrata of approximately neutral reaction and containing relatively little protein and ample mineral matter—media approaching the type of substratum on which the ascigerous stage occurred in nature, viz. stubble straw.

Morphology and Nomenclature.

Morphological Characters and Germination. The vegetative part of the fungus consists, at first, of hyaline hyphae, 3μ to 5μ wide, and sparingly septate. These increase in width, the septa become more numerous and the contents more granular, whilst a single oil globule (or group of two or three) develops at each pole of the segments. The hyphae branch freely, typically from near the ends of segments (Text-fig. 1, III and 2, I *c*), and become of a faint greenish and later distinct olive-green colour in mass. On nutrient substrata the hyaline mycelium produces bud-spores abundantly, these arising close to the septa or at the ends of terminal segments; each spore remains attached temporarily to the parent segment by a minute "neck," but after separation no sign of this connection is visible, as a rule. The bud-spores themselves produce daughter buds terminally (Text-fig. 3, III), the process being analogous to the budding of yeast cells, but without the production of alcohol. The bud-spores are at first hyaline, and later of faintly greenish tinge when they appear faintly olive-green in mass. Normally they show a single oil globule at each pole, but on some media the single globule is replaced by a group of two or three smaller ones. The mature bud-spores are elliptic-oblong in shape, and approximately 7·5 to 10·5μ



Text-fig. 1. *Dematium pullulans* (de B.); drawn from strain WI.

I. Mycelial hyphae with chlamydospores; (*a*) chlamydospores associated with thin-walled hyphae.

II. Type of chlamydospore associated with thin-walled hyphae.

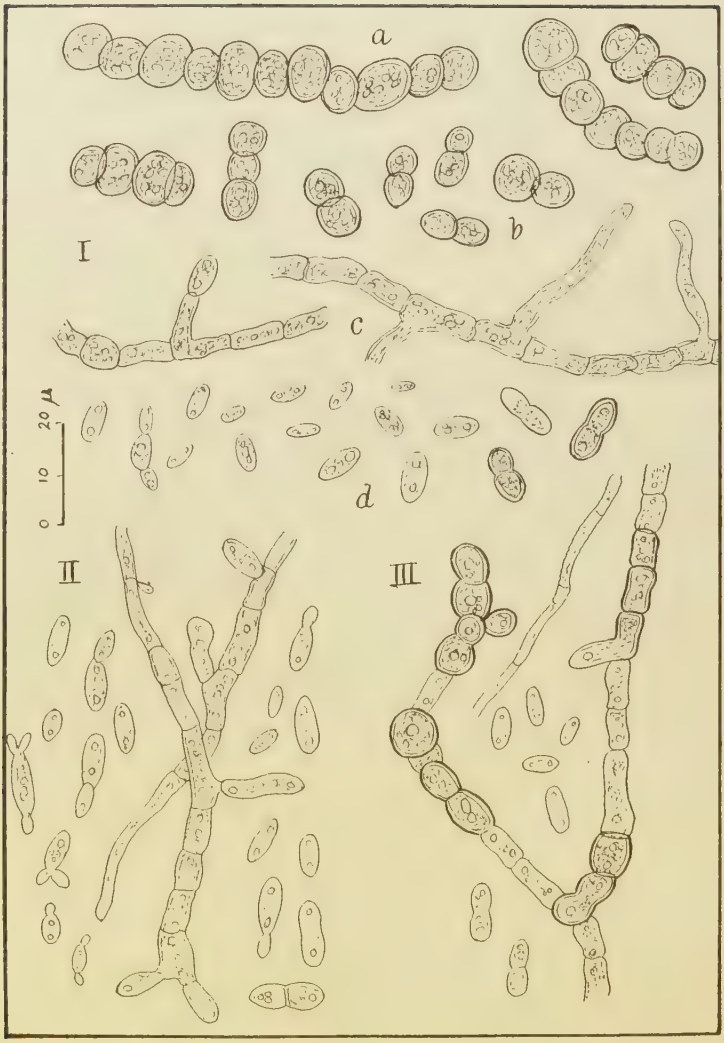
III. Mode of branching and production of bud-spores.

IV. Bud-spores; (*a*) budding, (*b*) becoming septate, (*c*) forming chlamydospores, (*d*) germinating in water.

long and 3·8 to 5·0μ wide. In older stages, many become one-septate, or show partial separation of the contents into two cells without a definite septum.

When the mycelial segments reach a certain size, and more rapidly on some media than on others, they become shorter and wider, their walls increasing in thickness and darkening in colour to olive-brown (Saccardo's olive), whilst a membranous envelope develops within and the contents show additional oil. These mycelial chlamydospores may occur singly, but are usually in series, though rarely do all the consecutive segments of a filament become so changed; intervening ones remain thin-walled and lose their contents to provide for the increase in the others. The chlamydospores vary in shape from oblong to spherical, the former measuring 10μ to 15μ by 9·5μ, and the latter up to 11μ in diameter. Smaller chlamydospores occur in association with hyphae which do not increase in size as described (Text-fig. 1, I and 2, III), and pseudo-chlamydospores are produced when the walls of ordinary mycelial segments become slightly thicker and darker; this combination of modifications constitutes the olive growth in tissues and the submerged laterals in culture media, and is the form which is scarcely distinguishable from Cladosporium. Chlamydospores of a third type are produced from bud-spores, which increase more or less in size, become two-celled, and develop thick outer, and membranous inner, walls. They differ in shape and size from the mycelial structures, and closely resemble some spores produced by *Cladosporium herbarum* (Text-fig. 1, IV, and 2, I (*d*)).

Bud-spores germinate in a few hours in water or nutrient solutions at room temperature (aver. 10° C.). From one or both ends an elongate-cylindrical germ tube emerges, and becomes a stout, hyaline hypha, septate at its proximal end and nearly as wide as the spore, but in its distal parts much narrower and sparingly septate. It produces bud-spores in the neighbourhood of septa. Bud-spores may arise from the germinating spore along with the germ tube or alone. Whilst germinating the spore swells considerably, and lays down a single transverse septum or shows a division of cell contents to two parts. Chlamydospores have been observed to germinate both in culture and in nature soon after their formation, merely by increasing the amount of available moisture at ordinary temperatures (Text-fig. 3, II); on the other hand, they have retained vitality for more than two years in cultures not completely dried out. In nutrient solutions the germinating chlamydospores yield clusters of bud-spores, some of which develop into hyphae, whilst with



Text-fig. 2. *Anthostomella pullulans* (de B.) comb. nov., showing the greatest observable differences from the *D. pullulans* of Text-fig. 1. Grown from ascospores as follows:

I. On Dox's agar, 7 days; (*a*) chlamydospores of mycelial segments, (*b*) other forms, (*c*) mode of branching, (*d*) bud-spore forms

II. On beerwort agar, 7 days.

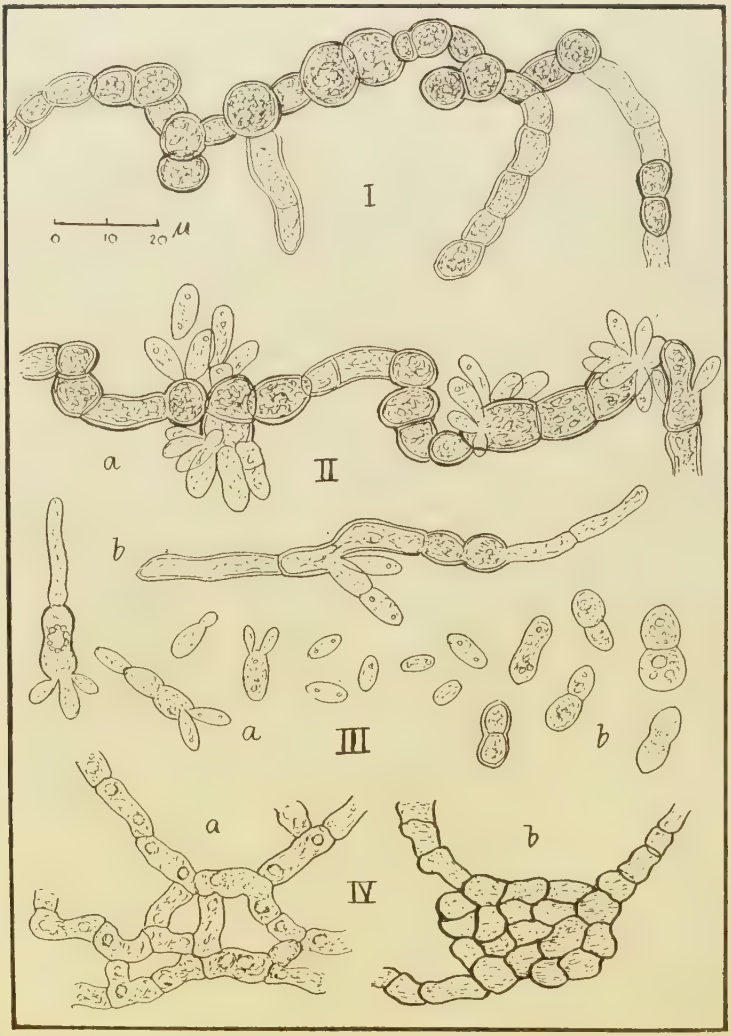
III. On wheatmeal agar, 7 days.

scant nutriment, as in water or on moist grain, a single, closely septate germ tube may be produced, this yielding hyphal branches and comparatively few and small bud-spores. The germ tube from a bud-spore penetrates a suitable substratum, and in a living plant ramifies in the parenchymatous tissues. When the tissues become exhausted the organism forms chains of chlamydospores and microsclerotia (Text-fig. 3, IV (*a*, *b*)), and in this form is visible as brown or blackish streaks in the dying or dead tissues. Growth was obtained from these resistant structures after exposure throughout a severe winter, the temperature at times being as low as − 15° C., whilst, on the other hand, they yielded the fungus in normal form after being in the laboratory for eighteen months. The organism, therefore, is well able to persist from season to season in its vegetative stages.

Strains of the Fungus. The morphological characters recorded above are common to all the strains, for microscopical differences between them are not clear. Thus, the forms AsI and WI, shown to be distinct macroscopically, are illustrated in Text-figs. 1 and 2, showing the greatest differences observable in much material examined; and it is obvious that, in general, these forms would pass undistinguished under the microscope. Cultures of these two strains, without any indication of their origin, were submitted to Mr J. Ramsbottom, of the British Museum[1], who stated that in his opinion both were *D. pullulans*. Now as the AsI culture was derived from a single ascospore (as were many exactly similar parallel cultures, p. 374) and was indisputably *D. pullulans*, it is evident that *D. pullulans* has an ascigerous stage. Further, it has been shown that AsI, WI, WII, CI, CII, and AsII are merely members of a series, no one member of which is distinguishable microscopically from its neighbour, so that all would, undoubtedly, be classed as *D. pullulans*. Therefore we must acknowledge a number of strains of this one fungus, and that this fungus has an ascigerous stage. Hence *D. pullulans* is a distinct entity, not a collective name for the conidial stages of several Ascomycetes, as suggested by Berlese (3) and Brefeld (4). Further, accepting the dictum that the diagnosis of a species must be based primarily upon structural characters, there is no justification for distinguishing these strains as species.

The Ascigerous Stage. The ascigerous stage occurred on wheat straw in autumn, appearing externally as a blackish fleck or small patch. The latter is a sclerotial structure projecting slightly beyond the surface of the straw, and perforated by ostioles; the structure is actually a clypeus,

[1] Correspondence 4. ii. 24 *et seq.* is gratefully acknowledged.



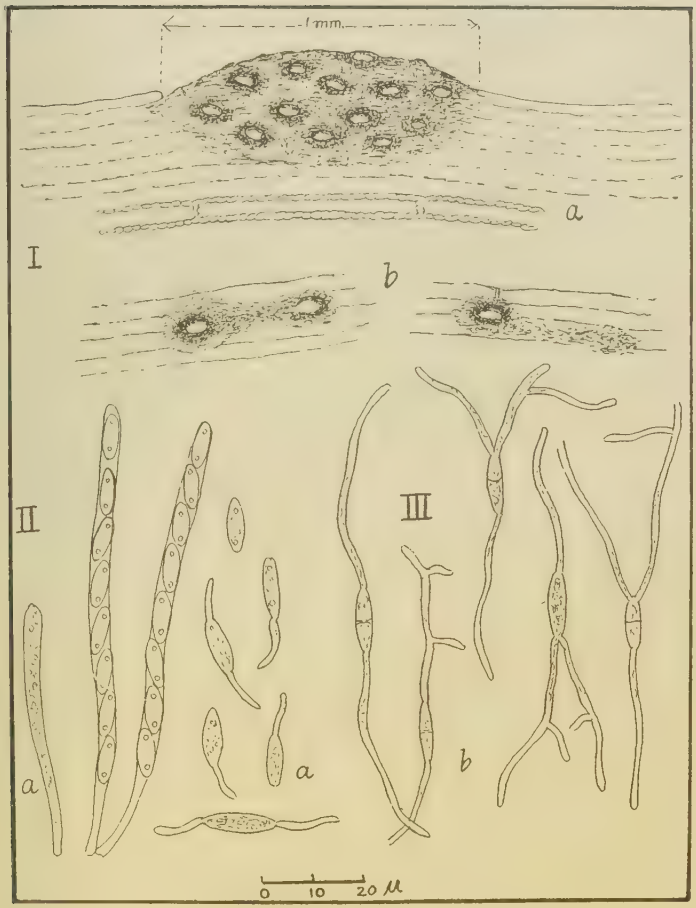
Text-fig. 3. *A. pullulans* (de B.) comb. nov. as recovered from wheat seedlings grown from inoculated grain (p. 389).

I. Mycelial hyphae with chlamydospores.

II. Germination of chlamydospores in water; (*a*) mycelial, *in situ* in tissues, (*b*) conidial.

III. Bud-spores; (*a*) sprouting and germinating, (*b*) forming chlamydospores.

IV. Formation of microsclerotia; (*a*) early stage, anastomosing hyphae, (*b*) later stage, microsclerotium.



Text-fig. 4. *A. pullulans* (de B.) comb. nov. drawn from original material on wheat straw.

I. Surface view (semi-diagrammatic) of clypeus; (*a*) large, (*b*) earlier, small, forms.

II. Asci with ascospores; (*a*) immature ascus.

III. Ascospores germinating in distilled water at 6° C. to 12° C.; (*a*) after 24 hours, (*b*) after 3 days.

as described later. The largest clypeus observed was about 1 mm. across and showed about a score of ostioles (Text-fig. 4, I (*a*)), but in the earlier stages one or two ostioles only with little surrounding sclerotial growth occur. The perithecia originate, few or many, in close proximity, in anastomosing hyphae just below the surface of the host tissue. The perithecium is globoid, and bounded by a many-layered, pseudo-parenchymatous tissue. Thickening of the cell walls of this tissue commences at the part nearest the host surface; an apical area, the future ostiole, remains unthickened but bordered by a band of the thickest walled cells, thus leaving the ostiole exposed to the exterior. The lowest parts of the lateral walls next thicken, and the thickening extends thence upwards to meet a downward extension from the apex, and more slowly along the base (see Text-fig. 5). Eventually the mature perithecium is surrounded by a dark brown wall several cells thick (Plate XX, fig. 6). Meanwhile thickening progresses from the apex of the perithecium along the hyphae in and immediately below the epidermis, so forming the clypeus. As shown in Text-fig. 4, I (*b*), the ostiole is distinct superficially before the clypeus is markedly evident, the latter increasing whilst the perithecium matures below. The union of these sclerotial structures of a number of adjacent perithecia gives rise to a single, comparatively large clypeus, which is visible to the eye. Both the clypeus and the perithecial wall are exceedingly brittle, breaking to fragments with even the weight of a cover-glass when examined under a microscope.

The ostiole has an extremely short, conical neck, is bounded by very thick-walled cells, and is elliptical in shape, with a long axis of approximately 30μ. The perithecium is a globoid cavity, occasionally somewhat triangular, with a thick, dark brown, brittle wall, sub-epidermal, at length erumpent, and perforate; the outer measurements are 160μ to 175μ vertically, and 200μ or more horizontally; the actual cavity measures transversely near the base 160μ, and 130μ vertically.

Asci arise from very slender, interwoven hyphae which form the base of the cavity. They are fasciculate, remaining in clusters by union at the foot end when the perithecium is broken in water. The mature ascus, from 85μ to 90μ in length, of which the spores occupy about 70μ, and 4μ in breadth, is an exceedingly delicate, hyaline membrane, evanescent after discharge of the spores, without pore, lid or apical thickening; the spores appear to be liberated by casual rupture or by dissolution of the membrane. There are no paraphyses. The ascospores are one-seriate, hyaline, elliptical, with an oil globule at each pole, unicellular, and measure from 8μ to 10μ by 3μ. They are remarkably

similar to the larger, thin-walled bud-spores (Text-fig. 4, II). In the process of germination nearly all ascospores develop a median, transverse septum (Text-fig. 4, III). The ascospores will germinate in the same



Text-fig. 5. *Anthostomella pullulans* (de B.) comb. nov.; perithecium (vertical section) approaching maturity; thickening of wall incomplete below; clypeal structure limited to the epidermal and sub-epidermal layers. (Semi-diagrammatic drawing with aid of camera lucida.)

autumn as produced, at any temperature between 6° C. and 23° C.; no tests have been made beyond these limits. In water the ascospore emits a germ tube from one or both ends, and at the same time becomes

two-celled (Text-fig. 4, III). The germ tubes are much narrower than the spore, hyaline, and soon produce lateral branches, after which septa are laid down. In a nutrient solution containing salts and dextrose, the germ tubes may bear bud-spores instead of branching, septa being developed later immediately below the bud protuberances. The mode of germination of the ascospore contrasts with the mode of growth of the very similar bud-spore, indicating a marked physiological difference between the two types. The lowest temperature which ascospores withstand has not been ascertained, but their vitality is not impaired by exposure of material bearing perithecia throughout a severe winter season. Exposure for three minutes to moist heat at 50° C. kills these spores.

Immature perithecia were formed on artificial media by three strains, and on wheat after inoculation with strain AsI, but their production was rare and casual, and efforts to stimulate this phase have failed up to the present. The first perithecia obtained in culture occurred on a beerwort agar plate poured with spores taken from several colonies of the CI strain soon after its isolation. Three weeks after pouring, being left at room temperature, the plates were nearly dried out, and the numerous colonies each about 1 cm. in diameter. At several places where the olive-black edges of two colonies met[1] there were black bodies the size of pin-heads. These were smooth and glossy, without aperture, and broke in water with very slight pressure on the cover-glass, whilst further pressure caused the exudation of a soft matter rich in oil globules. In two of the three specimens so examined the soft exudate contained immature asci, which were exact replicas of the immature asci found in the natural perithecia (Text-fig. 4, II). Two similar bodies were transferred whole to poured wheatmeal agar plates, and the last remaining body was crushed in water under aseptic conditions and used for dilution in beerwort agar for other plates. In every case typical *D. pullulans* was produced on the plates, without trace of other fungus, and beyond all doubt the *D. pullulans* obtained in the bud-spore state from cherry had yielded the ascigerous stage up to the formation of asci. Immature perithecia were obtained later in pure cultures on wheatmeal agar slants of the strains AsII, CI, and CII. All occurred singly, associated with a small amount of olive sclerotial formation near the surface of the medium, the whole being 0·5 mm. or a little more in diameter, slightly raised, black, and some with an apical opening. None contained asci, but the

[1] The possibility of a dioecious (heterothallic) nature of the mycelium here indicated awaits investigation.

similarity of the structures to the proved perithecia of CI indicated that they were likewise immature perithecia. Inoculation of the stems of growing wheat, by pricking in inoculum from strains AsI and WI, and covering the places with moist cotton-wool, yielded from more than thirty inoculations only two immature perithecia, these each having a small black clypeus just visible to the eye, at the point of puncture.

It will have been noticed that the ascigerous stage is associated with olive or black growth, and that AsI, itself derived from the ascigerous stage, gave the greatest amount of black growth throughout the cultures. The conditions most likely to favour the ascigerous stage, therefore, appeared to be those which retarded or inhibited bud-spore production (see p. 377). It was shown by Schostakowitsch(15) and von Skerst(16) that bud-spore production ceased in a 50 per cent. solution of dextrose or saccharose, and at a temperature of 30° C. to 31° C. Neither of these methods stimulated perithecial formation in the cultures, and culturing at the natural temperatures throughout the year, and on "hard" and "soft" media, also proved unavailing. It would appear that there is some factor influencing this type of sporulation, provided in nature, but yet to be discovered for application under controlled conditions. The facts ascertained indicate that the ascigerous stage must be extremely rare, being restricted to a few strains or produced intermittently only.

Nomenclature. The fungus is clearly a member of the *Sphaeriales*, but to which of its families must be decided according to the significance attached to the thickening associated with the perithecium. When two or three perithecia are in proximity the thickening of the mycelial hyphae on, within, and immediately below the epidermal layer, together with parts of the affected substratum form a thin clypeus extending laterally. It frequently extends to a depth of four or five cells of the cortex, then appearing present in an ill-defined, disjointed condition between the lower parts of the separate perithecia. This, however, is quite unlike a normal stroma, and further, the only growth connecting one perithecium with another is generally that in the epidermis and to some extent in the layer of cells immediately below it only. Therefore, it is impossible to consider the perithecia as "immersed in a stroma." In all its main characters the fungus agrees with those marking the family Clypeosphaeriaceae, given as follows by Rabenhorst(13):

> Perithecia sunken in the bark or leaf-parenchyma, without definite stroma, but covered by a so-called clypeus—a pseudo-parenchymatous, brown or black, somewhat shiny tissue, sometimes sharply limited, shield shaped, sometimes more dot-like and not sharply defined. Occasionally this pseudostroma is present on both sides, that

is, above and below the perithecium, or it surrounds the latter on all sides. Asci numerous with thickened apex. (Rabenhorst, in a footnote, adds that he does not consider this family to be a natural one, as the essential feature, the presence of the clypeus, is not constant.)

In the present scheme of systematic classification the Clypeosphaeriaceae is an accepted family, and as the fungus in question agrees in characters, except for thickened apex of the ascus, it must be included in this family. Of the genera comprising this family, Anthostomella has the following diagnostic characters, again quoting Rabenhorst, as taken from Saccardo (*Conspect. Gen. Pyren.* p. 8), Sylloge I:

Without stroma; perithecia sunken, covered by periderm, ostiole scarcely projecting, the characteristic rings around which give blackish specks, of carbonous or carbonous-leathery consistency. Asci cylindric, 8-spored, usually surrounded by paraphyses. Spores uniseriate, elliptic or oblong, unicellular, brown. (Footnote: This genus belongs on the whole to warmer regions, and few species occur in our country.)

As the presence of paraphyses is not an essential feature, the fungus under investigation differs from the characters of the genus only in having hyaline instead of brown spores. But this difference does not exclude the fungus from this genus and necessitate the erection of a new one, since in other instances a genus contains forms with differently coloured spores, and the modern view is that the single factor of colour is not to be regarded as a generic distinction. The organism, therefore, would be a species of Anthostomella. It bears some resemblance to *A. phaeosticta* (Berk.) Sacc., but has rather longer asci, hyaline spores, and no paraphyses. It does not agree with any other species, so must be added as a new one, and following the accepted practice it receives the specific name *pullulans*. Hence *Dematium pullulans* de Bary is the conidial stage of *Anthostomella pullulans* (de B.) comb. nov.

Pathogenicity of *Anthostomella pullulans*.

Whilst investigating the question of *Cladosporium herbarum* in relation to "thinning out" and "deaf ears" in wheat(2), the writer thought it desirable to extend the inquiry to the relation of *Anthostomella pullulans* to the same disease, first because of the former record that *A. pullulans* was a polymorphic form of *C. herbarum*, and secondly because these fungi, though now known to be perfectly distinct, are so frequently associated in nature. The usual mode of occurrence of *A. pullulans* suggested, from the first, the improbability of its having any decided parasitic capacity, neither has such been attributed to this

organism, but in order to render more complete our knowledge of this subject brief reference is here made to the experiments concerning its parasitism and pathogenicity. Two strains only were tested, AsI derived from the ascigerous stage, and WI from the bud-spore (Dematium) stage on wheat.

When the fungus was established artificially on the exterior of wheat grains, and these grains were grown under abnormal conditions yielding semi-etiolated seedlings in a moist atmosphere, the fungus passed from the grain into the living shoot in 66 per cent. of the plants. In the infected shoots it was present in the sheath of the basal leaf, where it could be seen in some specimens with a hand lens as brownish to blackish flecks or streaks, whilst after incubation such streaks were visible in most of the shoots. They were due to the chlamydospore stage which developed in the parenchyma between the vascular bundles. The fungus was present also in the tissues of some leaf-bases within the outermost sheath, and external to the tissues, *i.e.* lying between the sheaths (Text-fig. 3). The fungus recovered from such tissues agreed in all respects with the original when grown on similar media. It was evident that this organism could attack and exist in unhealthy, but still living tissues.

When wheat grain, prepared as for the foregoing trial, was grown in sterilised soil under normal conditions, in an unheated greenhouse, and outdoors in a "cage" plot, the resulting plants were not adversely affected. Other plants growing under the conditions named were inoculated by different methods in the aerial parts and allowed to grow on. Different sets of these latter plants were covered with glass bell-jars for one, three, and five days respectively after inoculation, and to this is attributed the occurrence of *A. pullulans* on the sheaths of some of the lower leaves, where it grew on the outer and inner sides and within their tissues. Such leaves, which in the ordinary way die back comparatively early in the life of the plant, would suffer loss of vitality speedily and considerably under the conditions imposed with the object of favouring the fungus, and the sheaths of these then afforded a suitable substratum upon which it was able to establish itself. But none of the younger and vigorous parts of the plants were attacked, the growth of the plants was not checked, and they came into ear and yielded as many and as heavy grains as did the control plants. Other features, such as the establishment of the fungus on exposed coronal roots, indicated that the organism could affect and develop on dead, dying or unhealthy parts without discernible harm to the host. One experiment illustrating this

is worthy of mention. The prepared pots of plants were supplemented in one season by blocks of young wheat plants dug and carried from a field crop. *A. pullulans*, applied to the aerial parts of these, developed abundantly on leaf-sheaths and straw, but it was found later that the plants, like many in the field, were affected with *Fusarium* or *Ophiobolus* or both, thus being unhealthy from the first. This experiment, at first thought useless, actually confirmed the conclusions drawn from the results of all the experiments.

The conclusions were that *A. pullulans* is essentially a saprophytic organism, with weak parasitic capacity, that is, it establishes itself on living tissues only when the latter are enfeebled by abnormal conditions, old age, other fungi, etc., but cannot attack sound, healthy tissues. It is not pathogenic to wheat, and is certainly not a cause of "thinning-out" and "deaf ears" in wheat crops. The nature and extent of its parasitism as ascertained for wheat is probably similar for other host plants.

Summary.

The fungus *Dematium pullulans* de Bary, is in its perfect stage *Anthostomella pullulans* (de B.) comb. nov. The mature ascigerous stage occurs in nature, and in immature form has been obtained in pure culture from both the conidium and the ascospore of the organism.

A. pullulans retains vitality through all periods of natural drought and cold in this country by chlamydospores and microsclerotia; the perithecial stage is not essential for its continued existence, and appears to be formed intermittently only.

Six strains of this one species, *A. pullulans*, are described, showing the extent to which variation by transitional stages may occur within the species.

The fungus is saprophytic, or weakly parasitic, but non-pathogenic, and is of promiscuous habit as regards host material.

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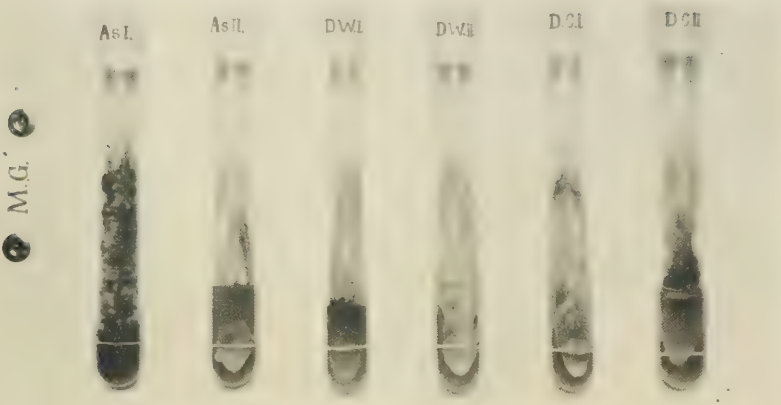


Fig. 1.

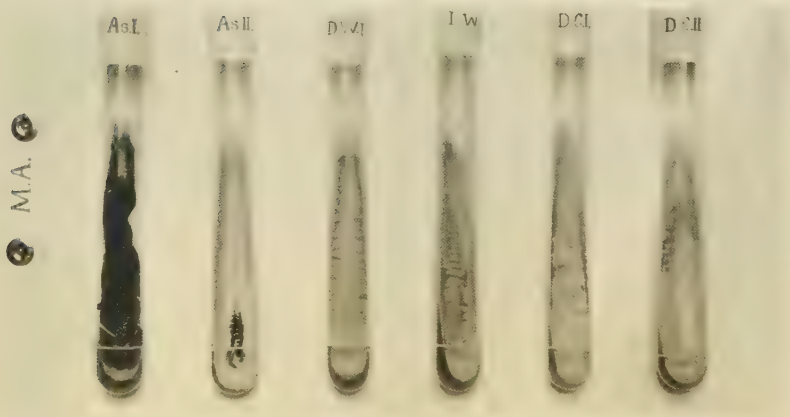


Fig. 2.

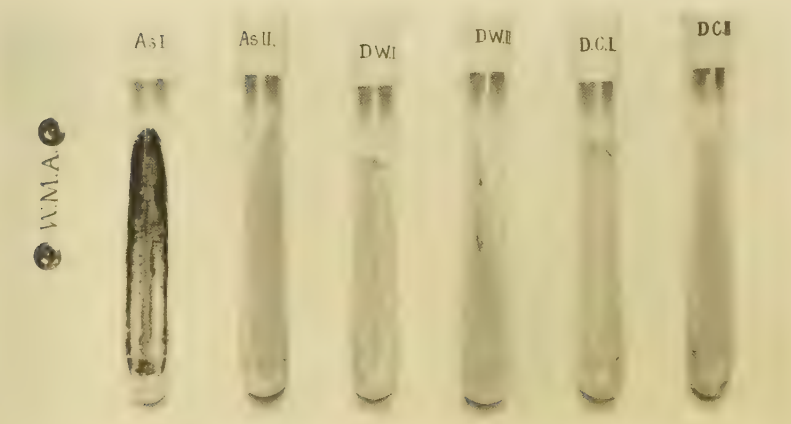


Fig. 3.

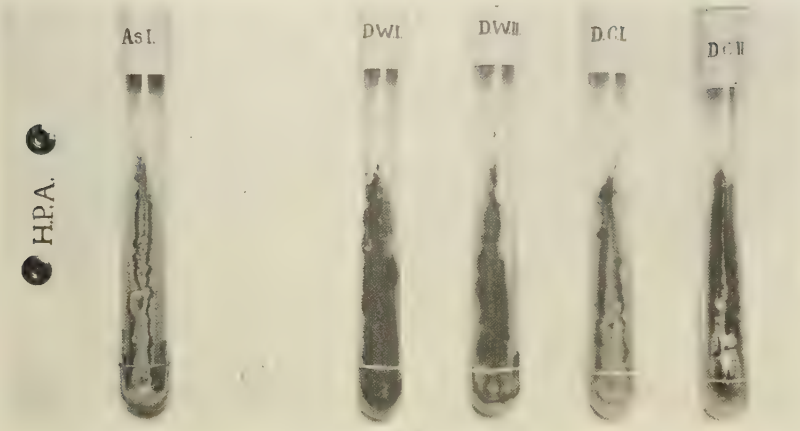


Fig. 4.

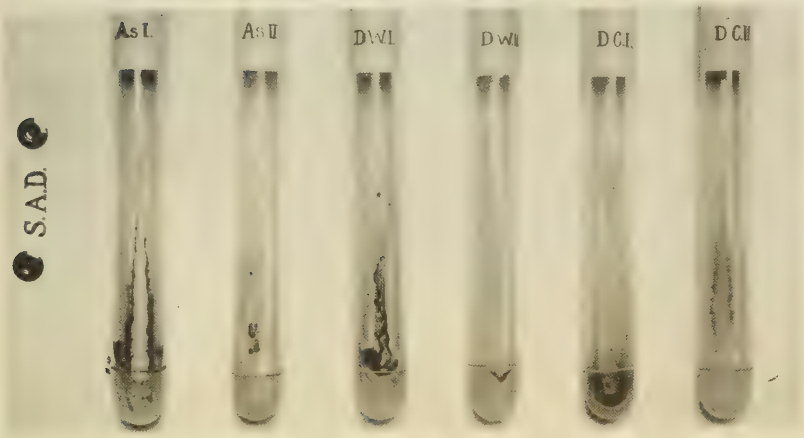


Fig. 5.

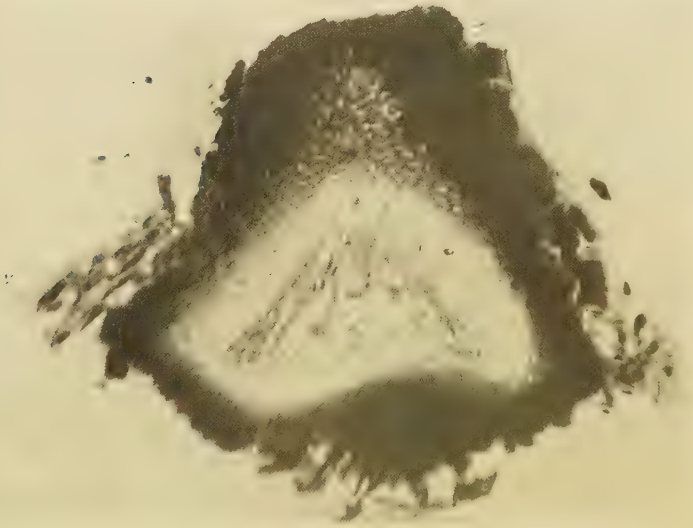


Fig. 6.

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EXPLANATION OF PLATES XIX AND XX

Figs. 1–5. Comparison of six strains of *Anthostomella pullulans* (de B.) comb. nov.

PLATE XIX.

Fig. 1. On malt gelatine.

Fig. 2. On malt agar.

Fig. 3. On wheatmeal agar.

PLATE XX.

Fig. 4. On hard potato-dextrose agar (AsII broken).

Fig. 5. On salts-dextrose agar.

Fig. 6. Perithecium (vertical section); portion of the clypeus in the epidermal layers on the left side broken away; normal appearance on the right.

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THE PRODUCTION OF POWER ALCOHOL FROM WASTE VEGETABLE MATERIALS, SUCH AS GRASS, STRAW AND HUSKS

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Failure to allow for the complex composition of most vegetable waste has been one of the principal causes of the slow progress shown by past experimental work on the utilisation of such waste as raw material for power alcohol production. Vegetable waste, for instance straw, sawdust or husks, is not composed primarily of cellulose, though it is frequently described as "cellulosic material," but contains more than 50 per cent. of other substances, notably of hemicelluloses, and of lignin, which differ fundamentally from cellulose in their chemical constitution and in their behaviour towards micro-organisms.

When it was decided to undertake an investigation of the conditions under which vegetable material could be converted into power alcohol, it became necessary to deal separately with the hemicelluloses, the lignified cellulose, and the unlignified cellulose.

As the problem of the conversion of the hemicelluloses offered the best prospects of a speedy solution, it was decided to approach this subject first. There were two avenues available by which this could be done, either:

(1) by subjecting the raw material to treatment with dilute acids, thereby converting the insoluble hemicelluloses into soluble monoses, suitable for power alcohol production by certain little known bacteria; or

(2) by initiating a search for bacteria hitherto unknown which were capable of converting hemicelluloses into power alcohol without a preliminary treatment of the raw material with acids.

Of the two methods the former was chosen in the first instance as it held out hope of a speedier solution than the latter. It was realised, however, that the second method, which may be described as a method for the direct fermentation of the raw material, might be of great importance under conditions and in districts where acid would be difficult

to procure. Steps were taken therefore to initiate research on this method by instituting a search for bacteria capable of fermenting hemicelluloses without preliminary hydrolysis.

It may be added here that this search proved successful in so far as it was possible to show that ethyl alcohol can be obtained by bacterial fermentation from hemicelluloses without having recourse to a preliminary hydrolysis of the raw material with acids.

The hemicelluloses present in most vegetable waste materials are composed primarily of pentosans which on hydrolysis yield pentoses, principally xylose. A method for the successful hydrolysis of hemicelluloses with acids had to secure not only the quantitative conversion of the hemicelluloses, but also the protection of the resulting xylose from destruction through caramelisation, a destruction to which xylose is particularly liable. A detailed account of the investigations carried out to establish the most favourable conditions for this hydrolysis was given in the Fourth Memorandum on the Production of Power Alcohol of the Fuel Research Board (1) and will not be reported here. It may be of

Table I.

Nature of material		Origin	Yield of fermentable copper reducing substances as pentoses %	Remarks
Local name	Botanical name			
Ekong grass	*Imperata cylindrica* Beauv.	Nigeria	21·4	
Eruwa grass	*Andropogon tectorum* Schum.	„	21·3	
Esun grass	*Pennisetum* sp.	„	19·9	
Gamba grass	*Andropogon Gayanus* Kunth.	South Africa	19·19	
„ „	*Hyparrhenia glauca* Stent.	„	18·4	
Jowar straw	*Sorghum vulgare* Pers.	India	19·9	
Maize cobs	*Zea Mays* L.	Kenya	21·3	Fermentation rather slow
New Zealand flax (residue)	*Phormium tenax* Forst.	Dorset	10·9	
Nile sudd	*Cyperus papyrus* L.	Egypt	19·5	
Rice straw Rice husks	*Oryza sativa* L.	Burma	19·9 13·0	Highly favourable for fermentation
Sisal hemp waste	*Agave sisalana* Perr.	Kenya	5·25	Highly favourable for fermentation, alone or with less readily fermentable materials
Wheat straw	*Triticum vulgare* Vill.	Dorset	18·3	
Wuchiyan Bera grass	*Ctenium elegans* Kunth.	Nigeria	20·8	
Yama grass	*Hyparrhenia rufa* Stapf	„	19·9	

Note. It is not possible to be certain of the exact identity of the plants in one or two cases.

interest to mention, however, that the method finally decided upon is based on a technique which enables the hydrolysis to be carried out at atmospheric pressures by simple steaming of the plant material, previously soaked in a 2 per cent. solution of sulphuric acid, followed by the extraction of the steamed straw with sterile water. Applying this method to a number of waste vegetable materials the following yields of fermentable, copper reducing substances were obtained.

Bacteria capable of converting pentoses to alcohol have long been known to the bacteriological literature. Frankland (2) obtained 6·93 per cent. of ethyl alcohol from xylose with Friedlander's *Bact. pneumoniae*. With *Bac. ethaceticus* he obtained from 11·2 to 12·3 per cent. of ethyl alcohol from arabinose. Another organism studied by Leichmann was stated by Tollens (3) to produce alcohol from xylose. Gayon and Dupetit's mannitol bacterium (4) was also claimed capable of producing ethyl alcohol from xylose. Particularly remarkable is Salkowski's statement (5) that certain bacteria, which are not specifically described by him, produce as much as 40 per cent. of ethyl alcohol from arabinose. The formation of acetone in addition to ethyl alcohol from pentoses was reported by Northrop, Ashe and Senior (6). The yields of alcohol obtained by them amounted to 18–20 per cent. with 4 to 5 per cent. of acetone, both calculated as yields on the pentoses fermented. Assuming a pentose content in waste vegetable material of, say, 20 per cent. Salkowski's and Northrop's types of pentose fermenting bacteria might be expected to give from 15 to 20 gallons of liquid fuel per ton of waste, the fuel in the case of Northrop's type containing 10 per cent. of acetone. Seeing that a ton of potatoes yields only from 18 to 22 gallons of liquid fuel, containing no acetone, the above yields from waste vegetable matter were quite favourable if it were found possible to devise a suitable technical method for the conversion. Attempts in this direction had been made by Northrop and his collaborators (7), by Peterson, Fred and Verhulst (8) and by Fred, Peterson and Anderson (9) and by Juritz (10). According to these authorities the fermentation of pentoses by Northrop's bacillus is comparatively slow, taking from 8 to 10 days to complete. To hasten the conversion of the pentoses, Northrop, Ashe and Morgan recommend the addition to the fermenting liquor of coke or beech shavings which, by extending the internal surface of the fermentation tanks covered by bacterial cells, was thought to be responsible for an increase in the fermentation activity of the bacteria. An increase in this activity is claimed by Peterson, Fred and Verhulst to be the result of the addition of peptone and di-sodium hydrogen phosphate to the liquor to be

fermented. These writers, as well as Northrop and his collaborators, emphasise the importance of maintaining the reaction of the liquid to be fermented within a range of hydrogen-ion concentrations of the *p*H values 6 to 9. For this purpose they add calcium carbonate to the fermenting pentose solution.

As Northrop's bacillus, in spite of its comparatively slow action, appeared to be the most suitable organisms known to perform the conversion of pentoses into alcohol, efforts were made to secure a strain of the type. This was made possible through the kind offices of the National Collection of Type Cultures, The Lister Institute, London, S.W.

As received, Northrop's bacillus, *Bac. acetoethylicus*, consisted of a culture of slender rods measuring, after staining with methylene blue, 0·4μ by 2 to 4μ with occasional cells reaching 7·5μ in length. Spores were formed subterminally and were egg-shaped measuring 0·8μ × 1·6μ to 2·0μ. Typical for the organism was the formation of granules of volutin in liquids such as potato mash, broth and pentose extracts. These granules were present in numbers of from 1 to 3 in each cell and became visible after about 48 hours' growth under favourable conditions.

The organism developed well in broth with or without the addition of carbohydrates, and formed a whitish pellicle on the surface and a slight turbidity of the liquid. It developed readily from single spores and cells and could therefore be isolated in pure culture without difficulty. When grown in liquid media containing glucose or other carbohydrates such as pentoses and when the reaction of the medium was maintained on the acid side of the neutral point a certain amount of gas was found to be given off. An analysis of the gas showed it to consist of carbon dioxide and hydrogen in the approximate proportions of 3 : 2.

When the reaction of the culture medium was maintained permanently on the alkaline side of the neutral point by successive additions of sodium or calcium hydroxide solutions, no evolution of gas was observed.

The surface colonies of *Bac. acetoethylicus* on ordinary agar were greyish and developed comparatively slowly. They did not exceed a diameter of 1 mm. after 48 hours' incubation at 37° C. Their margin was whole and sharply defined. Examined in transmitted light and at slight magnifications they showed a very characteristic appearance, as if the light were passing through a homogeneous transparent substance partly covered by torn and scattered remains of an opaque pellicle.

Gelatine was not liquefied.

The optimum temperature for growth of the organism was found to be between 38–42° C.

As to the nature and the quantities of volatile fermentation products produced by *Bac. acetoethylicus*, Northrop, Ashe and Senior (6) state that the organism produces formic acid, ethyl alcohol, propyl alcohol, butyl alcohol and acetone. After growing for 12 days at 40° C., in a food solution containing 2 per cent. of xylose, the organism yielded in their experiments 18 to 20 per cent. of ethyl alcohol, calculated on the sugar, and 4 to 5 per cent. of acetone; *d*-arabinose gave 12 to 16 per cent. of ethyl alcohol and 6 to 7 per cent. of acetone; glucose, 22 to 23 per cent. of alcohol and 9 to 10 per cent. of acetone. Fred, Peterson and Anderson (9) obtained from the copper reducing substances extracted from oat hulls 26·9 per cent. of alcohol and 12·7 per cent. of acetone, with 6·71 per cent. of volatile acids.

In his important treatise on the butyric acid and butyl alcohol bacteria, Donker (11) records the following yields of products from glucose fermented by *Bac. acetoethylicus*. Ethyl alcohol amounted to 31·2 per cent. and acetone to 9·1 per cent. In addition he found 2·6 per cent. of formic acid, 5·2 per cent. of acetic acid, 0·60 per cent. of 2 : 3 butylene-glycol and traces of acetylmethylcarbinol. The gas given off—the fermentation was conducted in the presence of 1 per cent. of calcium carbonate—consisted of carbon dioxide and hydrogen, the yield of the former gas amounting to 52·6 per cent. of the glucose fermented, that of the latter to 1·52 per cent.

In experiments carried out on hydrolysed wheat straw mashes kept throughout at a reaction equal to a *p*H value of 6·8, which corresponds to that obtained by the addition of an excess of calcium carbonate to the mash, the following fermentation products were obtained in the investigations recorded in this paper, after 7 days' incubation of the fermenting mashes at 42° C.

Ethyl alcohol	30·06 %	Calculated as percentages on copper-reducing substances fermented
Acetone*	1·71 %	
Acetic acid	1·77 %	
Formic acid	12·60 %	
Hydrogen	9·9 cc. per gm. of copper-reducing substances	
Carbon dioxide	7·1 cc. per gm. of copper-reducing substances	

* The low yields of acetone is ascribed to the unfavourable reaction of the medium in which the dissimilation of the pentoses proceeded.

In addition, a heavy dark brown "oil" of specific gravity 0·9970 and of boiling point 96° C. was found to collect in the distillate after the

last of the alcohol had passed over. Its nature has not been determined. It represented about 2 to 5 per cent. of the copper-reducing substances. Higher alcohols such as propyl and butyl alcohol were not detected in the distillates from the mashes.

Where the reaction of the mash was adjusted during fermentation to a figure on the alkaline side of the neutral point, the evolution of gas was no longer noticeable, both the hydrogen and the carbon dioxide appearing to enter into reaction with substances in the mash.

As regards yields of acetone the percentages obtained appeared to be variable. The attempts made to establish the reason lead to no very definite information. It was found that the acetone concentration was higher when hexoses were fermented by *Bac. acetoethylicus* than when pentoses constituted the major part of the fermented carbohydrates, and that the presence of infecting micro-organisms had a destructive action both on the formation of acetone and on the acetone already produced.

Table II gives the yields of acetone from mashes[1] containing rice starch and glucose, with the yields obtained from a wheat straw mash (xylose) included for purpose of comparison.

The fermentations were carried out at 36° C., 43° C. and 46° C. respectively, in order to compare the results with those reported by Northrop, Ashe and Senior (6) on the effect of the temperature on acetone production.

Table II.

Sample incubated at	Carbohydrate content of mash: Before fermentation	Carbohydrate content of mash: After fermentation	Yield of alcohol acetone in carbohydrates fermented	Percentage of acetone in alcohol acetone mixture
		Starch		
36° C.	2·0 %	Nil	22 %	25·3
43° C.	2·0 %	Nil	16·7 %	23·5
46° C.	2·0 %	0·385 %	10·9 %	5·7
		Glucose		
36° C.	2·0 %	Nil	22 %	11·7
43° C.	2·0 %	0·58 %	15·5 %	17·1
46° C.	2·0 %	0·39 %	5·68 %	—
		Xylose (Wheat straw mash)*		
36° C.	1·6 %	0·3 %	32 %	7·2
43° C.	1·6 %	0·1 %	32·5 %	7·5
46° C.	1·6 %	0·9 %	23 %	2·0

* Carbohydrate content of mash as pentoses.

[1] The media used contained 0·1 per cent. of peptone, 0·1 per cent. of di-potassium hydrogen phosphate, 2 per cent. of the carbohydrate to be acted upon and an excess of calcium carbonate.

The low total yields of volatile fermentation products from the starch and glucose samples are no doubt attributable to the unsuitable nature of the food solution used, since starch and hexoses are converted by *Bac. acetoethylicus* into volatile products in the usual proportions when present in vegetable extracts.

It should be pointed out that the increased acetone yields obtained from hexoses in the series quoted in Table II cannot be accounted for by assuming that the increase is due solely to a decreased production of alcohol in these fermentations. Not only would a loss of 33 per cent. of total volatile fermentation products in the series of experiments at 36° C. be insufficient to account for an increase in the acetone percentages from 7 to 25 per cent. but experience has shown that fermentations with *Bac. acetoethylicus* which have given low yields of alcohol have shown corresponding decreases in yields of acetone.

Before an attempt could be made to evolve a fermentation process for the conversion of pentoses to ethyl alcohol by *Bac. acetoethylicus* it was necessary to study the fermentation properties of the organism towards pentoses both under ordinary conditions of growth and with the introduction of the various precautions advocated by the American investigators.

Data on the yields of decomposition products obtained from pentoses (wheat straw) when fermented by *Bac. acetoethylicus* under ordinary laboratory conditions and in the presence of calcium carbonate have already been given. In order to determine to what extent these yields would be affected by the precautions recommended by the American investigators, experiments were taken in hand to ascertain whether an increase in the internal surface of the containers in which the fermentation was being carried out would affect the reaction favourably, and whether the presence of phosphates would hasten the conversion of pentoses into alcohol.

To determine the value of an increased internal surface, hydrolysed rice straw extract was fermented by *Bac. acetoethylicus* in flasks which had been previously filled with loosely packed, washed and sterilised cinders. Two separate flasks containing cinders were used and, as control, a flask of 3 litres capacity, but containing no cinders.

The results of these fermentations are shown in Table III.

It will be observed that the mash fermented as readily, if not more so, in the absence of cinders as in their presence, an observation which was confirmed in a second series where small pieces of marble had been used for increasing the internal surface of the container and in which a

mash prepared from hydrolysed *Andropogon Gayanus* straw was fermented.

Table III.

Sample of hydrolysed straw extract	Concentration of copper-reducing substance in extract (estimated as pentoses)		Duration of fermentation	Amount of alcohol-acetone mixture obtained on distilling the fermented mash; calculated as percentage of the copper-reducing bodies	
	Before fermentation	After fermentation		Originally present in the mash	Actually fermented
I. 4 litres of rice straw extract	2·0 %	0·42 %	6 days	14 %	17·7 %
II. 2 litres of rice straw extract	2·03 %	0·7 %	7 days	26·8 %	29·7 %
III. 2 litres of rice straw extract. Control	1·73 %	0·56 %	6 days	25·2 %	37·2 %

As regards the question of the value of an addition of food substances to the mash, an addition which some of the American investigators of the pentose fermentation recommended, it should be pointed out that the beneficial action might be due either to the buffering action of the added food or to an increase in the nutritive value of the medium. Müller (12), who studied the question of the functioning of peptone, showed that the concentration of 1 per mille was sufficient to satisfy the nitrogen requirements of his test organism and that any excess beyond this concentration functioned solely as a buffer. The concentration of 1 per mille of peptone (organic nitrogen) may be regarded probably as sufficient also for most other carbohydrate fermenting bacteria.

With 1·6 per cent. of protein present in wheat straw—the normal content—a sufficient amount of nitrogen for the normal development of *Bac. acetoethylicus* should therefore be present when using 7 to 8 per cent. of straw for the preparation of the mash.

In the case of wheat straw or rice husks the addition of peptone, or peptone and phosphates, did not seem to have any influence on the yields of acetone-alcohol obtainable, though a slight increase in the rate of fermentation during its later stages may have been noticeable in some cases. Table IV records some fermentation experiments illustrating the rate of fermentation of wheat straw and rice husk mashes.

In the case of other materials such as sisal hemp residue, rice straw and Nile sudd, the rate of fermentation was also found to proceed at a maximum without the addition of food substances, while maize cobs,

Table IV.

Mash prepared from	Percentage of acetone in liquid fuel	Concentration of copper-reducing substances in the extract (estimated as pentoses)		Duration of fermentation	Yield of alcohol-acetone mixture obtained calculated on the carbohydrates fermented
		Before fermentation	After fermentation		
Wheat straw	7·0	1·40 %	0·2 %	6 days	32 %
Wheat straw, and containing 0·1 % peptone	6·5	1·40 %	0·15 %	5 days	32 %
Wheat straw, and containing 0·1 % peptone and 0·1 % di-potassium hydrogen phosphate	7·0	1·40 %	0·15 %	5 days	32 %
Rice husks	8·0	1·60 %	0·1 %	4 days	33 %
Rice husks, and containing 0·1 % peptone	7·5	1·60 %	0·1 %	4 days	33 %
Rice husks, containing 0·1 % peptone and 0·1 % di-potassium hydrogen phosphate	8·0	1·60 %	0·1 %	4 days	33 %

Table V.

Sample of extract	Concentration of copper-reducing substances in extract (estimated as pentoses)		Duration of fermentation	Yield of alcohol-acetone mixture obtained on distilling the fermented mash; calculated on the carbohydrates		Percentage of acetone in alcohol mixture
	Before fermentation	After fermentation		Present	Fermented	
Sisal hemp residue	1·10 %	0·2 %	5 days	27·2 %	33·0 %	7·0
Maize cobs	1·57 %	0·78 %	11 days	14·6 %	30·4 %	3·0
Maize cobs + Sisal hemp residue equal parts	1·82 %	0·2 %	6 days	29·0 %	32·5 %	6·0
Maize cobs + Sisal hemp residue equal parts	1·58 %	0·15 %	7 days	32·5 %	35·9 %	7·0
Maize cobs + 20 % Sisal hemp residue	2·37 %	0·1 %	9 days	33·0 %	34·9 %	7·0

as may be seen from Table V, undoubtedly required additional nitrogen to secure a complete fermentation of their extracted carbohydrates.

It need hardly be emphasised that the use of peptone on a technical scale would be out of the question for economic reasons. Where additional food is required waste substances would have to be utilised, substances such as fresh grass with a fairly high nitrogen content and available in localities where the contemplated raw materials for alcohol manufacture exist.

In the case of maize cobs, sisal hemp residue might be adopted since mixtures of sisal waste and maize cobs have been found to yield highly suitable mashes giving a complete conversion of the large percentage of copper-reducing substances extractable from hydrolysed maize cobs. A series of fermentations illustrating this is recorded in Table V.

Another point on which great stress is naturally laid both by Northrop and by other American investigators of the pentose fermentation is the adjustment of the reaction of the liquid to be fermented to a suitable hydrogen-ion concentration. As such, Northrop, Ashe and Senior recommend a *p*H value of 8·0 to 9·0 for the growth and 6·0 to 8·0 for the fermentation by *Bac. acetoethylicus*.

To ascertain the optimum hydrogen-ion concentration for initial development and the subsequent fermentation two series of experiments were carried out. In the first, hydrolysed wheat straw, prepared and sterilised without the application of temperatures above 100° C., was fermented at a range of hydrogen-ion concentrations equal to a *p*H value of 5·2 to 9·2. Each of the samples within this range was inoculated with 1 per cent. of a 48-hour old liquid culture of *Bac. acetoethylicus*. Table VI indicates the rate of development of the organism in these samples.

Table VI.

Sample of wheat extract of a *p*H value of	Growth of *Bac. acetoethylicus* observed in the extract after incubation at 40° C. (hours)				
	24	48	72	96	120
5·2	–	–	–	–	–
5·5	–	–	–	–	–
5·74	–	–	–	–	–
6·13	–	+	+ +	+ +	+ +
6·59	–	+	+ + +	+ + +	+ + +
7·06	–	+	+ + +	+ + +	+ + +
7·45	+	+ +	+ + +	+ + +	+ + + +
7·76	+ +	+ + +	+ + + +	+ + + +	+ + + +
7·83	+ +	+ + + +	+ + + +	+ + + +	+ + + +
8·06	+ + +	+ + + +	+ + + +	+ + + +	+ + + +
8·53	+ + +	+ + + +	+ + + +	+ + + +	+ + + +
8·63	+	+ + +	+ + + +	+ + + +	+ + + +
8·73	–	+ + +	+ + + +	+ + + +	+ + + +
9·04	–	–	–	–	–
9·20	–	–	–	–	–

– = no growth + = slight development + + = distinct development
+ + + = fairly abundant growth + + + + = abundant growth

Here, as in all other cases, the measurements of hydrogen-ion concentration were carried out electrometrically. On a semi-technical scale test papers prepared by impregnation of filter-paper with a 0·4 per cent. alcoholic solution of phenol red were used in combination with red and blue litmus paper for the approximate estimation of the reaction of the fermenting liquid. The data thus established were subsequently confirmed by electrometric determinations.

Though initial growth of *Bac. acetoethylicus* occurred within the range of *p*H values from 6·13 to 8·73, it was most marked between *p*H 7·45

to 8·63 with a definite optimum round *p*H 8·0 to 8·53. This is a somewhat narrower range than that found by Northrop, Ashe and Senior and is less alkaline.

A study of the influence of the prevailing hydrogen-ion concentration of the mash on the fermentation activity of *Bac. acetoethylicus* when grown in pentose mashes was first initiated on a laboratory scale but had to be abandoned owing to the difficulties met with in adjusting the reaction of the mash under aseptic conditions and at sufficiently frequent intervals. Satisfactory data were obtained, however, on a semi-technical scale. The results of the three series of fermentations of wheat straw extracts recorded in Table VII have been compiled from the semi-technical scale fermentations. In series I the reaction was kept throughout the fermentation at a *p*H value of from 6 to 7, the mash never being allowed to become alkaline.

In series II, the reaction of the mash was adjusted to oscillate between the *p*H values 6·0 and 8·5 by occasional additions of sterile lime.

In series III the reaction of the mash remained alkaline throughout the fermentation, oscillating between the *p*H values of 7·5 and 8·5.

It will be seen that, though favourable for the initial development of the organism, a hydrogen-ion concentration equal to a *p*H value of 8·1 to 8·3 is unsuitable for the fermentation. The best reaction in this respect would appear to be one in which an alkaline reaction is established from time to time but at sufficient intervals to allow the mash to become acid.

The theoretical considerations arising out of these observations cannot be discussed in the present paper, but are obviously of considerable significance.

Before a technical method of utilising the hemicelluloses of waste vegetation could be elaborated for the production of alcohol, using *Bac. acetoethylicus* as the active agent in the fermentation, it had to be shown that it was possible to convert sufficiently high concentrations of copper-reducing bodies for the economic recovery of the alcohol to be possible. In all fermentation processes the recovery of the fermentation products represents a serious charge on the economics of the process and it is for this reason that the recovery of alcohol in distilleries is considered impossible in the case of mashes containing less than 1 per cent. of ethyl alcohol. To obtain mashes of an alcohol concentration of 1 per cent. or more it would be necessary to utilise extracts of waste vegetable mashes of a carbohydrate concentration of at least 3 per cent. since *Bac. aceto-*

Table VII.

Description of mash	Concentration of copper-reducing substances (estimated as pentoses) after 0 day %	1 day %	2 days %	3 days %	4 days %	5 days %	6 days %	Duration of fermentation days	Reaction of mash during fermentation	Yield of alcohol-acetone on copper-reducing substances fermented %	Percentage of acetone in alcohol-acetone mixture
No. 1. Wheat straw extract	1·54	1·36	1·20	1·0	0·85	0·7	0·6	6	Oscillating between *p*H 6·3 and 6·8	26	4
No. 2. Wheat straw extract	1·6	1·5	1·1	0·8	0·5	0·2	0·1	6	Oscillating between *p*H 7·8 and 6·3	33	8
No. 3. Wheat straw extract	1·5	1·4	1·0	0·9	0·9	0·8	0·78	6	Oscillating between *p*H 8·3 and 8·1	26	6·0

In each case the reaction was adjusted by the addition to the fermenting mash of a sterile lime suspension. These additions were given in the case of the first and the second experiments at 4-hour intervals in the case of the third at 2-hour intervals.

Table VIII.

Description	Concentration of copper-reducing substances (estimated as pentoses) after 0 day %	1 day %	2 days %	3 days %	4 days %	5 days %	6 days %	Duration of fermentation days	Reaction of mash during fermentation	Yield of alcohol-acetone on copper-reducing substances fermented %	Percentage of acetone in alcohol-acetone mixture
Wheat straw mash	3·0	2·7	2·0	1·5	0·9	0·5	0·15	6	*p*H 7·8 to 6·3	32	7
Rice	3·1	2·7	1·9	1·1	0·6	0·2	0·1	6	*p*H 7·8 to 6·3	33	7·5

ethylicus gives a yield of from 30 to 33 per cent. of alcohol-acetone mixture from the carbohydrates fermented by it.

The data collected in Table VIII show that it was possible to ferment mashes containing 3 per cent. of copper-reducing substances in the case of rice husks extract and wheat straw extract. Other suitable raw materials, though not actually examined from this point of view, may be assumed to behave similarly.

It is noteworthy that the fermentation in these cases of high concentration mashes proceeded at practically double the rate usually observed with more dilute extracts, an observation which, though theoretically anticipated, had not been hoped for in practice.

The various points ascertained in these series of experiments confirmed that *Bac. acetoethylicus* gives appreciable yields of ethyl alcohol and acetone from the carbohydrates extractable from such vegetable waste as rice husks, straw and grasses, and that the conditions governing this conversion are such that they can be safeguarded on a technical scale. Steps were therefore taken to devise a process for the large scale

preparation of power alcohol from such materials, incorporating in the process the advantages of the soaking method of hydrolysis devised for the purpose.

It had been found in the work carried out to devise a suitable process of hydrolysis that 6 hours' steaming of the raw material, in the presence of sufficient 2 per cent. sulphuric acid to render the material uniformly damp, resulted in a complete conversion of the insoluble hemicelluloses present to fermentable copper-reducing substances (xylose). If this period of steaming could be assumed to suffice also for the destruction of the normal microflora of the raw material, consisting of both spore and non-spore forming types, the necessary fermentation plant could be considerably simplified. The use of expensive pressure-resisting vessels for the separate sterilisation of the mash, prepared from the raw material, could then be dispensed with.

To ascertain whether a complete sterilisation of the raw material occurred during the steaming required for hydrolysis, a series of bacteriological analyses was made of the flora of various vegetable material before and after their hydrolysis by the steaming method.

For each analysis 1 gm. of the air dry, not hydrolysed, material was taken, and 5 gm. of the hydrolysed material, this being the weight corresponding approximately to 1 gm. of the original air dry substance. The quantity taken was mixed with 99 c.c. and 95 c.c. respectively of sterile physiological salt solution and the mixture shaken repeatedly for 2 hours. At the end of this time suitable dilutions of the various suspensions were plated out on agar and wort agar plates and the number of colonies appearing after 3 days' incubation at 30° C. was counted.

Table IX.

Type of material	No. of micro-organisms per gm. of raw material before hydrolysis
African grass No. 1	250,000
Andropogon Gayanus	880,000
Ctenium elegans	720,000
Ekong grass	3,500,000
Esun grass	4,000,000
Hyparrhenia glauca	124,000
Maize cobs	1,800
Rice husks	16,000
Rice straw	656,000
Sisal hemp residue	4,200,000
Wheat straw	4,800,000

Table IX records the numbers found in the types of raw material stated before steaming. At the end of the steaming period of 6 hours all the exposed samples of raw materials were found sterile. The whole of their microflora, including spore forming and non-spore forming rods, cocci, actinomycetes and fungus spores, had therefore been effectively destroyed.

Sterilisation under pressure might therefore be dispensed with in this part of the process on a technical scale. It had to be ascertained, however, whether the same would be possible in the case of the water used for extraction of the hydrolysed raw material and for the lime suspension required for the adjustment during fermentation of the hydrogen-ion concentration of the mash.

Though the prospect of being able to do so was favourable in view of Chick's(13) observations on the effect of alkalies on the destruction of bacteria by heat, it had to be determined experimentally at what hydrogen-ion concentration on the alkaline side of the neutral point micro-organisms, and notably spore forming types, could be destroyed with certainty at 100° C. or below.

The figures in Table X give the answer to this question and show that non-sporing bacteria (*Bact. coli commune*), cocci (*Micrococcus aurantiacus*), and spores (from *Bac. subtilis*), were destroyed after 5 minutes' exposure at 100° C.

Table X.

Reaction of the suspension of test organisms	Numbers of bacteria present in 1 c.c. of the suspension after exposure to 90° C.									
	Bact. coli		*Micrococcus aurantiacus*			Spores of *Bac. subtilis*				
	0 min.	5 min.	0 min.	5 min.	10 min.	0 min.	5 min.	10 min.	15 min.	20 min.
*p*H 7·1*	120,000	Nil	142,000	Nil	Nil	220,000	220,000	2,100	70	Nil
*p*H 8·17	120,000	„	142,000	„	„	220,000	80,000	—	80,000	14,000
*p*H 10·17	120,000	„	142,000	„	„	56,500	—	30,000	7,800	6,500
*p*H 12·08	120,000	„	142,000	„	„	56,500	47	1	4	Nil
*p*H 12·93	120,000	„	142,000	„	„	220,000	Nil	Nil	Nil	Nil

Reaction of the suspension of test organisms	100° C.							
	Bact. coli		*Micrococcus aurantiacus*		Spores of *Bac. subtilis*			
	0 min.	5 min.	0 min.	5 min.	0 min.	5 min.	10 min.	15 min.
*p*H 7·1*	120,000	Nil	120,000	Nil	220,000	1	Nil	Nil
*p*H 8·17	120,000	„	120,000	„	220,000	9	„	„
*p*H 10·17	120,000	„	120,000	„	56,500	10	„	„
*p*H 12·08	120,000	„	120,000	„	56,500	Nil	„	„
*p*H 12·93	120,000	„	120,000	„	220,000	„	„	„

* All except this suspension were buffered by the addition of 1 per cent. of sodium phosphate.

It was to be concluded from these experiments that if both the water used for extraction of the hydrolysed raw material and the suspension of lime required for adjusting the reaction of the mash could be given a minimum hydrogen-ion concentration equal to a *p*H value of 10·17 their sterilisation could be performed without the application of temperatures above 100° C. An adjustment of the *p*H value of the lime suspension to the figure required was found unnecessary since its concentration of 1 part of CaO to 9 parts of water showed a *p*H value of 12·6. Ordinary boiling was therefore more than sufficient in this case.

To raise the alkalinity and to secure sterilisation of the water used for extraction of the hydrolysed raw material, alkali would have to be added to it before heating in sufficient quantities to lower its hydrogen-ion concentration to a *p*H value of at least 10·17. This could be done by the addition of lime, a material which in any case would be required for the neutralisation of the acid remaining in the raw material after draining and pressing. In this way extraction and neutralisation of the extract could be performed in one operation and a saving thereby secured not only in the sterilisation of the water used for extraction but in the subsequent handling of the extract or mash.

Basing the technique on the principles already outlined and using a plant worked as far as possible on these principles, a number of raw materials have been fermented on a semi-technical scale, in lots of 100 to 110 lb. each. The yields of alcohol-acetone mixture thereby obtained are given in Table XI, expressed as gallons per ton on the air dry material.

Table XI.

Nature of raw material	Percentage of fermentable copper-reducing substances extracted by the soaking method of hydrolysis calculated as pentoses	Yield of alcohol-acetone mixture obtained, expressed as gallons per ton of raw material
Hyparrhenia glauca	18·4	18
Maize cobs	21·0	22·5
Pennisetum sp. (Esun grass)	16·7	17
Phormium tenax	11·0	10
Rice husks	13·0	14
Rice straw	19·9	19
Wheat straw	18·3	16

In addition to the above, the following raw materials have been fermented on a laboratory scale where they have given the yields of acetone-alcohol recorded in Table XII.

Table XII.

Nature of raw material	Percentage of fermentable copper-reducing substances extracted by the soaking method of hydrolysis calculated as pentoses	Yield of acetone-alcohol mixture obtained from raw material, expressed as gallons per ton
Andropogon Gayanus	20·9	20
Andropogon tectorum (Eruwa grass)	21·3	22
Sorghum stems (*Sorghum vulgare*)	17·0	17
Papyrus (air dry)	19·5	16·5
Sisal hemp residue	5·25	4·8
Sugar beet residues	28·8	24·7

Summary.

It has been established that the hemicelluloses of various waste vegetable materials can be hydrolysed by acids to yield extracts fermentable by alcohol forming bacteria.

A study has been undertaken of the conditions under which *Bac. acetoethylicus* Northrop can be utilised for the fermentation of the extract obtained by the hydrolysis of various vegetable materials and the conditions have been ascertained under which the fermentation may be technically applied.

A method is described for carrying out this fermentation without the application, for the preparation and sterilisation of the mashes, of temperatures exceeding those of boiling water.

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THE GERMINATION AND EARLY GROWTH OF WHEAT TREATED WITH COPPER CARBONATE AND TILLANTIN R.

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I. Introduction.

The importance of possessing accurate knowledge concerning the influence on growth of methods and materials which are used in disease control cannot be too strongly emphasised. Copper sulphate and formalin, by reason of their excellent fungicidal properties, achieved a wide popularity before it was realised that the benefit resulting from their use, unless the greatest precautions are exercised, is often largely counter-balanced by their injurious action on the grain. The adverse influence of these chemicals is no doubt often masked in field trials by reason of the fact, only recently established, that the fungus *Tilletia tritici* damages not only the fruit, but also retards growth in height, and under certain conditions is indirectly responsible for a poor stand in the field (15, 24). Thus it is possible for treated and untreated grain to make similar growth in parallel plots, the one suffering from chemical injury, and the other from the action of the parasite. Both types of injury are lost sight of if plots of untreated and uncontaminated grain are not included in the trial.

When the application of dry powders began to supersede the older methods, the superior growth of treated grain repeatedly caused comment, and led to the belief that the chemicals employed possessed the power of stimulating growth.

In a series of experiments conducted at the Aberystwyth Plant Breeding Station in the seasons 1923–25 for the purpose of testing the

relative efficiency of various fungicides, striking differences were evident in the establishment, vigour of growth, and final grain yield of treated and untreated samples. The superior growth appeared to be related to the degree of disease control rather than to the influence of any specific chemical (23). The authors decided therefore to study as a separate problem the influence on growth of *Tilletia tritici*. The work has shown that the adverse influence of this fungus is greater than was formerly supposed, and furnishes an explanation of the reduced vigour of untreated samples in the above trials (24).

The present paper deals with the germination and early growth of uncontaminated grain treated with copper carbonate and with an organic mercury preparation similar in composition to uspulun, which was used as a solution in some of the previous trials.

Both chemicals gave in one experiment slightly higher germination figures, but the early growth of treated and non-treated grain was in all cases closely similar, and the statistical data collected give no certain evidence of "stimulation" by the chemicals employed[1].

The results are not without a distinct positive value, since they indicate that the two fungicides, though not "stimulating," are at least not injurious to the plants.

II. HISTORICAL.

An extensive survey of literature bearing on the problem of plant stimulants was published by Brenchley in 1914 (3)[2]. Recent agricultural journals give evidence of an increased interest in the subject. This may be attributed in part to the work of Popoff on "cell-stimulation" (19), in part to the mass of experimental work, which followed the introduction of the new fungicides containing organic mercury compounds.

Popoff, working with cereals and other economic plants, obtained an increase in yield amounting not infrequently to 40 or 50 per cent., by soaking the seed with solutions of manganese and magnesium salts. He considered the results sufficiently constant to justify the introduction of the methods to practical agriculture, but later workers have not always obtained positive results with similar solutions (5). A detailed

[1] These conclusions relate only to these chemicals applied in the manner stated. No attempt has been made to test them in smaller or greater doses. Concentration of the chemical is known to be a critical factor in other examples of stimulation (3).

[2] It is unfortunate that the revised edition of this book which appeared recently (1927) does not include papers bearing on the question of "stimulation" by treating seed with copper carbonate, magnesium salts or organic mercury compounds.

review of such literature will not be given here since the authors have not included salts of manganese or magnesium in their experiments. The work is mentioned because it appears to have influenced the interpretation of experimental data dealing with the growth of seed treated with uspulun and other fungicides. A tendency may be noted in recent European publications to regard the terms "chemical stimulants" (*Reizchemikalien*) and "steeping materials" (*Beizchemikalien*) as synonymous. From a survey of recent literature such a point of view appears to the authors not to be justified.

Early work on the fungicidal properties of chlorphenol-quicksilver solutions and their influence on seed germination was summarised and discussed by Remy and Vasters in 1923 (21). The conclusion was reached that the favourable influence on the energy of germination which not infrequently followed treatment was due to the fact that seed infected by fungi (in particular *Fusarium* sp.) had generally been used for the tests. Healthy seed when treated did not show the same improvement. Extensive experiments were carried out by these authors with healthy seed of wheat, barley, oats, rye, and other species, the results of which confirmed the above conclusion. They showed however that solutions, which in field experiments proved to be strongly fungicidal, exerted no *adverse* effect on the germination of non-contaminated seed. The optimum concentration of solutions and the necessary period of steeping were the subject of detailed experiment.

It will be convenient to consider the more recent papers in three groups[1].

1. *Investigations which deal primarily with the efficiency of certain materials as fungicides for specific diseases, and which contain evidence strongly suggestive of a marked superiority in the germination, growth or yield of treated seed.* The comparison in such experiments is between contaminated grain, treated and untreated, and from the context it

[1] The majority of the papers quoted deal with several fungicides differing widely in chemical composition. The review of literature would be too bulky were all these to be considered, and the writers have confined the discussion to germisan and uspulun (wet and dry). The latter substance has appeared under a variety of names, Tillantin, Uspulun-Trockenbeize, Bayer dust (Britain), but the firm (I.G. Farbenindustrie A.-G., Höchst a. M.) has decided that in future their liquid disinfectants shall be described under the name Uspulun and that the name Tillantin shall be employed for similar compounds which are to be used in the dry state. (Re-naming of seed disinfectants, *Rev. Appl. Myc.* VI, 278, 1927.)

Uspulun-Trockenbeize is said to have increased the energy of germination in the case of certain horticultural crops (28). The results are interesting, but since no reference is made to the presence or absence of disease organisms the papers are not included in the present review.

is permissible to conclude that the so-called "stimulation" is at least in part a fungicidal effect. References to the following crops and parasites may be quoted: wheat (*Tilletia* sp.) (12, 16, 17, 6); oats (*Ustilago* sp.) (8); rye (*Calonectria graminicola*) (9, 25); flax (*Botrytis* and other fungi) (26).

2. *Investigations relative to the influence of steeping solutions on seed selected as reasonably free from fungus spores.* These require fuller consideration.

In 1925 Senf (27) published the results of extensive experiments with wheat, showing the influence of different methods of treatment on the energy of germination and the first stages of growth. The methods were tested on several winter wheat varieties, seed of which was obtained from plants grown under uniform conditions. The varieties are described but no reference is made to the conditions under which the seed was harvested, or to its freedom from fungus spores. The germination tests were carried out in sand, and parallel tests in boxes of soil supplied data relative to the height of the first leaf from soil level, together with the green and dry weights of "shoots" per 100 plants at the end of 15 days. The comparison is made between seed untreated and seed soaked in water and in different concentrations of uspulun for varied periods of time. Considering the influence of uspulun, tested on three wheat varieties, it is evident that treatment has improved the energy of germination but not the total germination of the seed. This is true when treated lots are compared with untreated seed or with seed soaked in water, but in this case the difference is much less. Improvement is also seen in the data for length of leaf, and for green and dry weights at the end of 15 days. The following figures quoted from Table D indicate the type of result obtained:

Dry weight in gm. per 100 plants—untreated 0·556; water 2 hours, 0·632; 0·1 per cent. uspulun, solution 2 hours, 0·696.

Similar results were obtained with germisan and tillantin B. Discussing the germination tests, the author notes the fact that the development of roots was more vigorous in samples treated with uspulun. The conclusion is finally reached that it may become a profitable agricultural practice to steep even healthy seed in certain solutions before sowing.

An extensive series of germination experiments followed by field trials of seed treated with various chemicals including uspulun and germisan was carried out by Becker (1). The energy of germination in wheat, barley, rye and several other economic species was improved by

certain concentrations of uspulun and germisan and by a number of inorganic salts, but in no case did the apparent stimulation continue to show in the early growth stages of the plant, and treatment did not result in increased yield. Becker concludes that the apparent stimulus to germination given by certain preparations is the result of a fungicidal action.

The action of uspulun, dry and in solution, on wheat (presumably spore-free) is discussed by Mencacci(13). Laboratory germination tests were carried out and grain treated by different methods was sown in duplicated plots in the field. The wet treatment lowered the germination slightly in laboratory tests but no perceptible influence was detected in the field trials. Treatment with the dry powder did not lower appreciably the germination of the grain, but it did not give any indication of "stimulation."

Germination tests on filter paper and in soil were carried out by von Bittera(2) with wheat treated with six dry fungicides and six different solutions. Photographs taken on the ninth and twelfth days illustrate the relative growth in soil, showing that whereas certain treatments (formalin and copper sulphate) have retarded growth, none has stimulated it. The slight difference in favour of certain treatments (including uspulun) shown at the beginning of the germination tests was rapidly lost.

Von Feilitzen(5), who included uspulun in his experiments with solutions of magnesium chloride, obtained a slight increase in grain yield as the following figures show.

Oats. Untreated 100; soaked in water 97 ± 1·3; uspulun 0·25 per cent. solution 2 hours, 109 ± 1·3.

Barley. Untreated 100; soaked in water 115 ± 9·4; uspulun 0·25 per cent. solution ½ hour, 118 ± 1·3.

Kempski(10) has reported on the stimulating effect of uspulun (0·25 per cent. solution) on various cereals, recording the fact that plants from treated seed were more drought resistant than those from seed soaked in solutions of formalin or copper sulphate. The experiments did not include a comparison with seed soaked in water and it is not clear from the context that the samples under test were free from disease organisms.

An adverse effect on germination, early growth and rate of respiration was recorded by Czarnowski(4), as following the treatment of a spring wheat with a 0·5 per cent. uspulun solution. This result, which appears to be contrary to general experience, may possibly be due to the use of a stronger solution than that usually advocated.

3. *Investigations relative to the influence of fungicides on seed of low germination capacity.*

A series of experiments conducted by Krosby (11) primarily with the object of comparing filter paper and sand as media on which to conduct germination tests with cereals, provides data showing the influence of germisan (30 minutes' immersion in 0·25 per cent. solution) and uspulun (1 hour's immersion in 0·4 per cent. solution) on badly harvested seed infected with species of *Fusarium*, *Aspergillus*, *Mucor* and bacteria. The trials are particularly interesting since they include not only laboratory tests but also carefully conducted field trials. Treatment of damaged grain was followed by higher germination, more vigorous growth, and an increased final yield. Samples of oats, barley, wheat, and rye gave similar results. Treated samples showed a higher correlation than untreated between laboratory and field germination figures, especially when the filter paper method of testing was used[1].

Experiments on similar lines were carried out by Gadd (7) with barley and autumn wheat of low vitality. The barley is described as showing characteristic symptoms of storage injury but was not attacked by typical parasitic diseases. The tests were conducted in sterilised sand and in non-sterilised soil at 10, 15 and 20° C. The technique of testing is described. Treatment consisted in soaking samples for different periods in 0·25 per cent. germisan solution, and drying them on filter paper for 1 hour before planting. The comparison is made with dry seed, not with samples soaked in water. Treated and untreated samples showed no difference in germination in sterilised sand at either temperature, or in soil at 20° C. At 10° C. and 15° C. in soil the germination of the treated series was distinctly superior to the control, and this difference was confirmed by the average height and weight of 100 normal plants at 10° C. The author comments on the fact that disinfecting with germisan did not check the development of *Penicillium* on dead grain.

A similar experiment was carried out with autumn wheat, free from *Fusarium*, but suffering from storage injury. Samples soaked in water for different periods were included with the dry control, for comparison with others treated with 0·25 per cent. uspulun solution (5, 10, 15 up to 60 minutes' soaking), tutan, dry uspulun, manganese chloride (1·0 per cent.), manganese sulphate (0·75 per cent.), and potassium permanganate (0·5 per cent.). The percentage increase produced by certain treatments is shown by the following figures quoted from Table II.

[1] The first part of the investigation proves that sand is a better medium than filter paper for testing cereals.

	Water	Dry uspulun	Wet uspulun
Total germination	106–112	113	113–119
Dry weight of normal plants	100–101	105	104–108
Dry weight of all plants	107–112	106	117–122

It has seemed essential to review the literature in some detail since the experimental results are so closely linked with questions of technique. The conclusions may be summarised as follows:

Solutions of uspulun, by controlling certain diseases which are seed-borne, may increase germination, growth and yield. The improvement in energy of germination which may follow the application of uspulun to healthy grain does not usually extend to later growth[1].

Badly harvested samples of cereal seed respond to treatment with uspulun. This is probably a fungicidal effect. There is at present no definite evidence for regarding uspulun as a plant stimulant.

Literature relating to the use of copper powders was reviewed in earlier papers (22, 23). Morettini (14), who alone has studied the influence of copper carbonate on grain free from fungous spores, concluded that the slight increase (2–3 per cent.) in germination of treated lots was due to the toxic effect of the powder on soil organisms. In this connection it is interesting to note that Thomas (29) found the same substance effective when applied to soil for the control of tomato diseases due to *Phytophthora* sp.

Pritchard (20) found that the germination of grain already injured by thrashing, was lowered slightly by dusting with copper carbonate. Sound grain suffered no injury, and this appears to be the general opinion of those who have employed this chemical for the control of bunt.

III. Experimental Data.

The method adopted in the present series of experiments is essentially the same as that described in a previous paper dealing with the influence of *Tilletia tritici* on the growth of wheat (24). The samples under study, in this case healthy grain treated and not treated with the two fungicides, copper carbonate and tillantin R, were sown either in wooden boxes measuring $21 \times 12 \times 7$ in. or in red earthenware pans measuring $10 \times 10 \times 4$ in. In each experiment the units were arranged on a chequer-board system and kept under uniform conditions in cold or temperate glass-houses. The grain was sown at regular spaced intervals

[1] The authors recognise the fact that under certain soil or climatic conditions accelerated germination may be a very important factor in the establishment and therefore final yield of a crop; see (30).

and covered with the same depth of soil. The watering was carried out as uniformly as possible.

Notes on growth and counts to determine the rate of germination and final establishment were made at intervals, and the height of each individual plant was obtained at the close of the experiment by measuring from soil level to the apex of the longest leaf. In order to obtain data on dry weights, the contents of each box or pan was turned out and the plants were separated as carefully as possible from the soil. After recording the number per unit, and in some cases counting the tillers, each plant was divided into "shoot" and "root" by cutting across the axis at the level of the scutellum. The roots from each unit were washed, dried in an oven, and weighed. The shoots received the same treatment except that it was not always found necessary to wash them before drying.

Details concerning the grain; the place, date and duration of the experiment; the soil used and the number of replications of treated and untreated samples, are given below for the separate experiments. The results are summarised in Tables I–IV.

Exp. 1. Copper carbonate and tillantin R.

Variety. Standard Red (Ca 720).

Place, date and duration of experiment. Temperate glass-house. May 17th, 1926, to June 10th, 1926.

Replications and soil used. 8 boxes. 104 grains per unit. Partially sterilised soil[1]. The sample of grain used for this experiment was the produce of plants grown at the Station in 1925 from grain treated with copper carbonate before sowing. Harvesting and thrashing operations were carried out with due precautions against contamination by spores of *Tilletia* or other parasitic fungi.

Exp. 2. Copper carbonate.

Variety. Square-head Master (Ca 728).

Place, date and duration of experiment. Cold glass-house. November 1st, 1926, to February 1st, 1927.

Replications and soil used. 19 boxes. 60 grains per unit. Partially sterilised soil.

Exp. 3. Copper carbonate.

Variety. Square-head Master (Ca 728).

[1] The primary object of using partially sterilised soil was to avoid wireworm attack. Soil saturated with water was heated to 85–90° C. in a brick oven and maintained at this temperature for approximately 12 hours.

Place, date and duration of experiment. Cold glass-house. November 1st, 1926, to February 8th, 1927.

Replications and soil used. 18 earthenware pans. 25 grains per unit. Partially sterilised soil.

The grain sown in Exps. 2 and 3 received the following treatment. A 1 lb. sample from a seed firm was carefully inspected and all grains were discarded which showed visible marks of injury by thrashing or weathering. The selected portion of the sample was soaked in formalin solution (1 part formalin to 480 parts of water) for five minutes at 11° C., drained thoroughly and left for four hours in filter paper moistened with the same solution. The sample was then washed in several changes of tap-water and spread out in a thin layer to dry. Two days later, half the sample was shaken in a tin with copper carbonate and the excess powder was removed by sieving.

Exp. 4. Tillantin R.

Variety. Square-head Master (Ca 767).

Place, date and duration of experiment. Cold glass-house. February 24th, 1927, to April 22nd, 1927.

Replications and soil used. 12 boxes. 60 grains per unit. Rotted turf from the farm not partially sterilised.

The seed used for this experiment was sterilised in formalin solution by the method described above. Cracked or discoloured grains were rejected in this case after sterilisation. The selected sample was thoroughly mixed and divided into two portions, one of which was shaken with tillantin R and then sieved to remove excess of the powder.

Exp. 5. Tillantin R.

Variety. Hen Gymro (Ca 484).

Place, date and duration of experiment. Cold glass-house. October 26th, 1927, to December 16th, 1927.

Replications and soil used. 12 boxes. 60 grains per unit. Partially sterilised soil.

Exp. 6. Tillantin R.

Variety. Hussar (Ca 609).

Place, date and duration of experiment. Cold glass-house. October 26th, 1927, to December 13th, 1927.

Replications and soil used. 15 earthenware pans. 25 grains per unit. Partially sterilised soil.

The two samples of grain used in Exps. 5 and 6 were obtained from plants which had been grown in a glass-house. The heads were thrashed by hand, and it was judged unnecessary to sterilise the grain before starting the experiment. The tillantin R was applied to one-half of each sample as in previous experiments.

Table I.

Showing establishment and early growth of wheat treated with copper carbonate and tillantin. (Ref. C 150. IV.)

Exp. 1. Variety: Standard Red.

Treatment	Average germination per box	Average dry weight of shoots per box in gm.	Average dry weight of shoots per 100 plants
A. Untreated	97·8 ± 0·847	7·135 ± 0·300	7·297
B. Copper carbonate	98·0 ± 0·474	7·120 ± 0·267	7·266
C. Tillantin R	98·1 ± 0·341	6·984 ± 0·271	7·119

IV. Discussion of Results.

1. *Copper carbonate.* In Exps. 1 and 3 the germination and growth of the treated and non-treated grain were remarkably uniform. No visible difference between the two series appeared at any time during the experimental period, and the final yield data confirm this uniformity (Tables I and II). In Exp. 2 the copper carbonate series showed on the whole a very slight superiority and was judged by unbiased observers to be the better series. When the data are analysed it appears that the slight inferiority of the untreated series is mainly due to the poorer germination. The final figures for the establishment of the two series were 57·8 and 54·4 per unit, giving a significant difference of 6 per cent. in favour of the grain treated with copper carbonate. The same series is slightly superior in tillering, height, and dry weight data, but the differences are not mathematically significant. The authors hesitate to regard the superior growth as an expression of "stimulation" by copper carbonate, since the same seed gave completely negative results in a parallel experiment (No. 3) conducted in the same glass-house over the same period of time. They suggest that the difference, chiefly

Table II.

Showing establishment and early growth of wheat treated with copper carbonate. (*Ref.* C 180.)

Exps. 2 and 3. Variety: Square-head Master (Ca 728).

Treatment	Average germination per box or pan	Average number of tillers per plant	Average height from ground level to apex of longest leaf in cm.	Average dry weight of shoots per box or pan in gm.	Average dry weight of shoots per 100 plants in gm.	Average dry weight of roots per box or pan in gm.	Average dry weight of roots per 100 plants in gm.
Exp. 2. Boxes							
A. Untreated	54·4 ± 0·377	2·48	23·21	5·416	9·968 ± 0·490	1·179	2·172 ± 0·059
B. Copper carbonate	57·8 ± 0·194	2·56	23·78	6·152	10·639 ± 0·396	1·361	2·358 ± 0·063
Exp. 3. Earthenware pans							
A. Untreated	23·9	1·75	21·50	2·472	10·36	0·786	3·38
B. Copper carbonate	24·3	1·73	21·00	2·462	10·14	0·792	3·25

Table III.

Showing establishment and early growth of wheat treated with tillantin R. (*Ref.* C 186. I.)

Exp. 4. Variety: Square-head Master (Ca 767). Exp. 5. Variety: Hen Gymro (Ca 484). Exp. 6. Variety: Hussar (Ca 609).

Treatment	Average germination per box or pan Mar. 11th	Average number of tillers per plant	Average height from ground level to apex of longest leaf in cm.	Average dry weight of shoots per box or pan in gm.	Average dry weight of shoots per 100 plants in gm.	Average dry weight of roots per box or pan in gm.	Average dry weight of roots per 100 plants in gm.
Exp. 4. Boxes							
A. Untreated	55·3 ± 0·537	4·82	25·81	16·32 ± 0·200	28·70 ± 0·249	7·31 ± 0·192	12·86 ± 0·373
B. Tillantin R	58·8 ± 0·187	4·53	25·86	17·13 ± 0·212	29·10 ± 0·319	7·45 ± 0·252	12·66 ± 0·433
Exp. 5. Boxes							
A. Untreated	59·8	—	21·36 ± 0·164	3·39	5·65 ± 0·157	0·23	0·384
B. Tillantin R	59·8	—	20·59 ± 0·160	3·11	5·19 ± 0·149	0·23	0·385
Exp. 6. Earthenware pans							
A. Untreated	24·3	—	18·33	1·125	4·57 ± 0·119	0·144	0·586 ± 0·016
B. Tillantin R	24·5	—	18·35	1·245	5·08 ± 0·134	0·115	0·469 ± 0·012

Table IV.

Summary of results with treated grain given in terms of the untreated at 100.

	Exp. 1	Exp. 2	Exp. 3	Average
Copper carbonate				
Establishment	100	106	102	103
Dry weight of shoots per 100 plants	100	107	98	102
Dry weight of roots per 100 plants	—	108	96	102
Average height	—	103	98	100

	Exp. 1	Exp. 4	Exp. 5	Exp. 6	Average
Tillantin R					
Establishment	100	106	100	101	102
Dry weight of shoots per 100 plants	98	101	92	111	100
Dry weight of roots per 100 plants	—	99	100	80	93
Average height	—	100	96	100	99

expressed in total germination, is due to the control of an unknown factor (a fungus?) which was present in the soil[1].

2. *Tillantin R.* The four experiments with tillantin R gave no distinct visible evidence of superior growth in either treated or control. In each experiment the two series made very uniform growth. In regard to the final statistical data, Exp. 4 shows a difference in establishment which is probably just significant. The treated sample gave a germination of 58·8 plants per unit as against 55·3 for the control, an increase of approximately 6 per cent. In tillering, height, and dry weights the results for the two series are closely similar.

In Exp. 6 the dry weight of roots per 100 plants shows a 20 per cent. decrease in the treated sample, a result which appears to be significant. The obvious difficulty of obtaining reliable data on the weight of roots from plants in soil, and the exceptionally low figures obtained for the weight of roots in this experiment suggest however the need for caution in accepting this one result as a proof of the injurious action of tillantin.

Considering the data as a whole the authors conclude that the results show no definite evidence of injury resulting from the application of either tillantin or copper carbonate to the particular wheat samples under test. They do not consider that the slight improvement in one experiment only is sufficient evidence for regarding these compounds as capable of stimulating plant growth. These results are in harmony with

[1] On several occasions when the plants were taken up a search was made for the sown grain which had failed to produce a plant. Such grain was not infrequently covered by a growth of *Penicillium* sp., and it is possible that this fungus was a contributing cause to low establishment. It is interesting to find *Penicillium glaucum* given as the reason for low germination results in field trials with wheat in Australia (18). See also Gadd (7).

the conclusions of those workers who have carried their studies beyond the early stages of germination and have taken precautions to use healthy seed.

Reviewing their previous trials in the light of these results, the authors consider that the retarding influence of bunt was largely responsible for the remarkable differences between the growth of treated and non-treated seed. The evidence as a whole of all the trials that have been conducted, together with the results quoted from the literature under review, appears strongly to suggest that the fungicidal properties of substances like copper carbonate and tillantin may have a distinctly beneficial effect on brairding by inhibiting the growth of organisms present on the seed or in the soil. It is highly probable moreover that the influence of micro-organisms on the germination and early development of seedlings, more especially in the case of samples relatively weak or mechanically injured, has not been sufficiently appreciated; seed treatment with the dry fungicides deserves further consideration from this point of view.

Summary.

From a review of recent literature the authors conclude that the supposed "stimulating effect" of such substances as uspulun, tillantin, and copper carbonate, is in most cases clearly to be attributed to the fungicidal properties of these compounds.

The authors have conducted critical experiments to test the influence of copper carbonate and tillantin R on several varieties of wheat, taking precautions to eliminate as far as possible disease-producing organisms. Each compound improved the percentage germination in one experiment, but the trials as a whole gave no definite evidence of "stimulation." The conclusion is reached that these chemicals should not at present be included in the list of plant stimulants.

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ENGLISH-GROWN PYRETHRUM AS AN INSECTICIDE. I

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Introduction.

The use of certain species of pyrethrum (*Chrysanthemum*) for insecticidal purposes has been known for a considerable period of time; Dalmatian, Persian and Caucasian insect powders consist of the ground flowers, respectively, of *Chrysanthemum cinerariaefolium* Trev., *C. coccineum* Willd., and *C. marschallii* Ascher (syn. *Pyrethrum roseum* Bieb.)[1], though there appears to be some doubt as to whether the two latter are distinct species.

Chrysanthemum cinerariaefolium has been grown in Dalmatia and Japan for a number of years and its cultivation has steadily spread to almost all parts of the world. Excellent accounts of the history, character and cultivation of this plant, with a description of some of the work carried out to ascertain the nature and composition of its insecticidal principle and means for detecting its adulteration are given in Juillet's

[1] The Insecticide and Fungicide Board of the United States Department of Agriculture recognises these three species as those from which genuine insect powder is made.

monograph(5) and in the paper of MacDonnell, Roark and Keenan(6). Both of these works contain full bibliographies. It would therefore serve no useful purpose to refer here at any length to the literature of the subject, and the reader interested is referred to the above publications.

The cultivation of pyrethrum has become a fairly extensive practice in many countries. It is a matter of surprise therefore to find that only spasmodic and half-hearted attempts to raise this valuable plant have been undertaken in this country. It is recognised that the economic possibility of growing pyrethrum will depend upon the yield, upon whether it can be kept free from disease and whether, under our climatic conditions, the crop can be harvested and dried at an economic cost. The present investigation was undertaken to study the growing of pyrethrum for a period of years in this country with particular reference to the type of soil most suitable for its growth, and to the possibility of the occurrence of variations in toxicity in the flowers grown in different localities. Other points requiring study were the keeping qualities, the variation, if any, of toxicity with size of flower-head, and whether or no any specificity of toxic action is shown.

The authors wish to record their indebtedness to the authorities of the following Stations for their kind collaboration in growing plots of pyrethrum: Department of Agricultural Education, Worcester; Research Station, Long Ashton; Isle of Ely Demonstration Plot; University College, Reading; South-Eastern Agricultural College, Wye; East Malling Research Station; The Horticultural College, Swanley; Royal Horticultural Society, Wisley; The Farm Institute, Sparsholt, Hants; Seale Hayne Agricultural College, Newton Abbot; University College of Wales, Aberystwyth; Experimental Station, Scilly Is..

Experimental Cultivation of Pyrethrum.

A preliminary report on the experimental cultivation of pyrethrum in England appeared in the *Journal* of the Ministry of Agriculture(3) and dealt with the results of the work up to the close of 1926. The origin of the experiments was there described, and it will be sufficient for the purposes of the present paper if a brief summary of the earlier work be given.

Origin of seed. Thanks to the generosity of M. Vaysière, of the Station Entomologique de Paris, and of Messrs Joensson and Co., of Kobe, seed of pyrethrum, *Chrysanthemum cinerariaefolium*, both of European and Japanese origin, was obtained in 1924 and 1925. The European seed was produced at the Station Viticole at Lausanne, where experiments in the

cultivation of pyrethrum have been in progress for some years. The Japanese seed was of the standard commercial quality used for growing crops in Japan, where pyrethrum production has been carried out on a large scale for a much longer period. Two different races of pyrethrum were thus involved in the experiments, and, as was to be expected, considerable differences between them were discovered. The plants from the Swiss seed showed evidence of selective propagation in the relative uniformity both of habit of growth and of cropping, whereas the plants from Japanese seed were very diverse in both respects and suggested that no attention is given in Japan to the production of special strains of the plant. Although the Swiss plants were more uniform in character than the Japanese, they nevertheless showed considerable variation among themselves, and if habit of growth should prove to be correlated with toxicity of flowers, the strain should be capable of much further improvement at the hands of the plant breeder.

As soon as seed of the two strains was available, the kind co-operation of the authorities of a number of experiment stations was secured, and finally eleven plots of Swiss pyrethrum and nine of Japanese were planted. Owing to the fact that the Swiss seed was small in amount and arrived earlier than the Japanese, while the latter germinated badly, it was not possible to arrange that at each station there should be plots of both Japanese and Swiss plants, but sufficient stations are growing both races to enable a fair comparison to be obtained.

Soil. European accounts of pyrethrum growing invariably point out that the plant when growing wild is found on the poor calcareous soils of the Dalmatian region, and not unnaturally its cultivation on such soils is usually recommended. It is less clear, however, that the range of soils capable of growing good pyrethrum has been sufficiently explored, and therefore, in initiating the present experiments, it seemed wise to include a variety of soil types. In the present paper insecticidal tests are described with pyrethrum from different plots and the type of soil on these plots is briefly indicated as follows:

Harpenden	Clay with flints
East Malling	Light loam over greensand
Seale Hayne (Newton Abbot) ...	A sticky Devonian sandstone
Scilly Is. (St Mary's)	Light sandy granite soil
Sparsholt	Light calcareous loam
Swanley	Light thin loam on chalk
Wye	Chalk (patches of light loam)

The above list shows that a variety of soil textures have been obtained, although it is probable that so far as these particular stations

are concerned most of them show no marked lime deficiency (with the possible exception of the Scilly Is. and Newton Abbot). The subject will be discussed further when the results of tests with the 1927 crop are available.

Cultivation. Pyrethrum is a perennial which under English conditions requires rather more than a year before it comes into full bearing. Seed sowing may thus take place either in the autumn or spring, and the first crop will be secured in the second summer after sowing. Under Harpenden conditions, to which the following notes chiefly refer, the best results were obtained by sowing in autumn, the young plants being wintered in cold frames and planted out in the following spring; sowing in the open proved less successful, and since the plants gave little trouble in the seed pans or when pricked out in cold frames, it seems undesirable. In planting out, the French instructions were followed approximately and the young plants were set out at a distance of 18 in. × 18 in. By the following autumn the plants were beginning to touch and when the first crop was taken the ground was completely covered, so that small annual weeds were entirely suppressed and it was only necessary to remove by hand occasional large weeds, such as sow thistle and the larger grasses, which had succeeded in rising above the crop. In the winter there is considerable dying off of the old foliage, especially the outer leaves, which gives the crop a rather poor appearance at the end of winter, but as spring advances new shoots are developed and a healthy foliage is rapidly regained. It would seem that the pyrethrum plants are hardy under English conditions as they have withstood, without apparently suffering, the winters of 1925–26 and 1926–27, which were fairly representative; and this was the case even under very trying soil conditions, such as where the crop was planted in heavy wet clay. In regard to the duration of a plantation under English conditions, the oldest plot is one from Swiss seed, which was planted out in April 1925. Since then two harvests have been taken and in general appearance the plot looks good for at least another harvest. In France, a plantation is expected to last from eight to nine years, but it is improbable that so long a duration would be obtained in England.

Harvest. Although both leaves and flower-stems contain the insecticidal principles in small quantities, it is the flower which is chiefly of value. There is, however, no uniformity of opinion as to the exact stage at which the flower should be cut. In commercial circles it has become the established convention to regard as good samples only those containing flower buds in the almost closed condition. The results of certain

investigations in France and elsewhere suggest, however, that this convention is without foundation, open flowers being as toxic as almost closed buds. Since a considerably greater weight of crop can be obtained from a given area if harvest is delayed until the flowers are nearly open, it was considered desirable to test flowers cut at different stages, and details of this experiment appear elsewhere in this paper. So far as the majority of plots was concerned, however, an endeavour was made to cut flowers when the petals had fully developed but before they had flattened out. Local conditions—weather, labour, etc.—interfered with this endeavour in several cases, and as a result the samples from the different stations showed considerable variation—some consisting of almost closed buds as approved commercially and others of practically fully open flowers. (The experiment referred to above will show that except in so far as the total weight of crop is concerned this variation appears to be more or less immaterial.)

Harvesting consists of cutting the flowers with a convenient length of stalk (about 8 in.) and then removing the flower-heads either before or after drying—which is carried out by exposing the crop, spread in a thin layer, first out-of-doors (if there should happen to be any sunshine) and subsequently in a ventilated building. After drying for about six weeks the flower-heads can be safely stored in metal bins or tins. In regard to yield, considerable variation is shown by the different plots, but it is not proposed to discuss this subject, or indeed the economic possibilities of pyrethrum growing, in any detail until more information is available. It may, however, be of interest to mention that at Harpenden 1 rod of Swiss pyrethrum yielded 5 lb. of dried flowers in 1926 and 2 lb. 12 oz. in 1927 when cropped for the second time, whereas in 1927 1 rod cropping for the first time gave 3 lb. 5 oz. The average yield of all "Swiss plots" in 1927 was 3 lb. 14 oz. per rod. A conservative estimate of the yield in France is given as $2\frac{1}{2}$ lb. (approximately) per rod, and it is clear that English yields compare with this not unfavourably.

Experiments with *Aphis rumicis* L. (Black bean aphis).

The method used for the determination of the toxicity values of plant extracts to *A. rumicis* has already been described (10, 11). The essential features are the preparation of the extracts in a constant way, the preparation of the different concentrations of the extracts by dilution with an aqueous solution of saponin or soap, the spraying of the dilutions upon the insects under constant conditions, and the subsequent

classification of the effects under the headings "unaffected," "slightly affected," "moribund" and "dead."

A slight departure from the usual method of preparing the extracts was made, owing to the fact that the samples of pyrethrum flowers were not ground to the same condition of fineness as many of the other plant materials previously investigated. Instead, therefore, of soaking the ground flowers in absolute alcohol and filtering through muslin, the flowers were soaked in a known volume of alcohol for several days, with repeated shaking, allowed to settle and the clear supernatant liquid diluted. Throughout these experiments a 0·5 per cent. solution of saponin in water was used for dilution.

In its effects upon *Aphis rumicis*, pyrethrum is in a class by itself; its action is extremely rapid, producing a state of profound narcosis, which at the higher concentrations results in death; at lower concentrations, the narcotic action may wear off and after a period of time, which appears to depend upon meteorological conditions, the insects often partially and sometimes wholly recover from its effects. This necessitates examination of the insects for several days after spraying and renders classification under the headings "moribund" and "dead" somewhat difficult. It must therefore be understood that under these two headings are collected those sprayed insects which under the conditions of our

Table I.

Toxicity of pyrethrum to A. rumicis.

(Showing recovery with time.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Derivation of seed and part of plant	Concentration in terms of part of plant gm./100 c.c.	After 24 hours				After 48 hours				After 72 hours			
		N	S	M	D	N	S	M	D	N	S	M	D
Flowers from Swiss seed, Harpenden grown	0·25	—	—	—	10	—	—	—	10	—	—	—	10
	0·1	—	—	2	8	—	—	4	6	—	1	4	5
	0·05	—	—	3	7	—	3	6	1	7	1	1	1
	0·025	—	1	6	3	6	1	3	—	7	1	2	—
Stalks from Swiss seed, Harpenden grown	5·0	—	—	—	10	—	—	—	10	—	—	4	6
	2·5	—	—	—	10	—	—	3	7	—	3	3	4
	1·0	—	3	5	2	4	3	2	1	4	1	2	3
	0·5	4	1	3	2	4	3	—	3	4	1	2	3
Flowers from Japanese seed, Harpenden grown	0·5	—	—	—	10	—	—	—	10	—	—	—	10
	0·25	—	—	—	10	—	1	5	4	—	2	2	6
	0·1	—	—	3	7	—	3	6	1	—	4	2	4
	0·05	—	2	3	5	6	1	2	1	6	1	—	3
Control 1	0·5 % saponin	10	—	—	—	10	—	—	—	7	2	—	1
Control 2	0·5 % saponin	10	—	—	—	10	—	—	—	8	1	—	1

experiments were apparently moribund or dead after being kept for 2 or 3 days. The technique adopted does not allow of observations being continued much longer than this, because some of the unsprayed control insects then normally begin to show signs of failing.

Some examples of experiments showing this tendency towards recovery (which has only been observed by us with a few materials other than pyrethrum) are given in Table I.

In the subsequent tables, we have, for ease of comparison, expressed the moribund and dead together as a percentage of the number of insects sprayed, but owing to the difficulty of classification referred to, we do not lay stress on this figure as an accurate numerical estimate of the toxicities of the various extracts. The detailed figures in the tables form the most reliable basis of comparison.

The observed tendency towards recovery is not a property which invalidates the practical use of pyrethrum. It must be taken into account in detailed experiments on the comparative toxicities of different samples; but, under practical conditions, higher concentrations than the minima giving 100 per cent. "moribund" and "dead" under the conditions of our experiments would be employed. As will be seen, our results demonstrate that pyrethrum flowers have a very high toxicity indeed to certain insects.

Toxicity of pyrethrum grown at different centres.

The air-dried samples received from the various centres were ground, extracted with alcohol and diluted to known concentrations (expressed in the tables as percentages of the plant material) and sprayed upon adult wingless females of *A. rumicis*, reared under standard conditions, insects of the same size and age only being used. As in previous experiments, 10 insects were sprayed at a time. Control tests were carried out with 0·5 per cent. aqueous solutions of saponin and dilutions of absolute alcohol in 0·5 per cent. aqueous solutions of saponin. Alcohol is not toxic at concentrations considerably higher than those used in the dilutions of the pyrethrum extracts. After spraying, the insects were kept under observation until the controls showed signs of failing. The results obtained with the flowers grown in 1926 at different stations are set out in Table II. The figures for a sample of flowers grown in France are also included, the tests in each case being carried out in 1927 (June to August). The moisture content of all samples tested was determined by drying in an electrically-heated drying oven at a temperature of 104° C.

Table II.

Toxicity of pyrethrum flowers grown at different stations to A. rumicis.

(Harvest 1926: tested 1927.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Station	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
Harpenden from Swiss seed	14·8	0·5	—	—	—	100	100
		0·25	—	—	10	90	100
		0·1	15	10	20	55	75
		0·05	70	10	5	15	20
		0·025	70	10	20	—	20
Seale Hayne from Swiss seed	15·1	0·5	—	—	—	100	100
		0·25	—	—	—	100	100
		0·1	—	30	30	40	70
		0·05	40	40	20	—	20
Sparsholt from Swiss seed	14·2	0·5	—	—	—	100	100
		0·25	—	—	15	85	100
		0·1	25	35	30	10	40
		0·05	65	20	10	5	15
		0·025	80	20	—	—	—
Scilly Isles from Swiss seed	14·8	0·5	—	—	—	100	100
		0·25	—	—	—	100	100
		0·1	—	—	40	60	100
		0·05	—	10	75	15	90
		0·025	—	40	60	—	60
Swanley from Swiss seed	14·2	0·5	—	—	—	100	100
		0·25	—	—	5	95	100
		0·1	—	—	15	85	100
		0·05	—	10	60	30	90
		0·025	80	20	—	—	—
Wye from Swiss seed	14·3	0·5	—	—	—	100	100
		0·25	—	10	5	85	90
		0·1	20	5	30	45	75
		0·05	65	15	10	10	20
French grown	14·5	0·5	—	—	—	100	100
		0·25	—	—	—	100	100
		0·1	—	—	10	90	100
		0·05	—	—	20	80	100
		0·025	60	—	30	10	40
Harpenden from Japanese seed	15·2	0·5	—	—	—	100	100
		0·25	—	10	50	40	90
		0·1	—	30	60	10	70
		0·05	60	10	20	10	30
Sparsholt from Japanese seed	13·6	0·5	—	—	70	30	100
		0·25	—	—	90	10	100
		0·1	—	30	60	10	70
		0·05	70	10	20	—	20
Swanley from Japanese seed Dried at 50–65° F.	13·4	0·5	—	—	—	100	100
		0·25	—	10	10	80	90
		0·1	10	10	50	30	80
		0·05	50	40	10	—	10
East Malling from Japanese seed	14·8	0·5	—	—	—	100	100
		0·25	—	—	70	30	100
		0·1	60	10	30	—	30
		0·05	70	30	—	—	—
Wye from Japanese seed	13·2	0·5	—	—	30	70	100
		0·25	—	—	10	90	100
		0·1	60	10	—	30	30

Inspection of Table II shows that the samples grown from Swiss seed at Harpenden, Wye, Sparsholt and Seale Hayne have about the same toxicity, failing to kill 90–100 per cent. of the insects at a concentration rather less than 0·25 per cent. The samples grown at Swanley and in the Scilly Isles and the sample derived from France are apparently somewhat more effective. This result is in all probability significant for these samples under the conditions of our experiments, but it is improbable that small differences of the order found would be detectable in practice. For practical purposes all the samples may be considered to have approximately the same toxicity to *A. rumicis*.

The results obtained with the flowers grown from Japanese seed at the various stations are also all of the same order and show them to be about as toxic as samples grown from Swiss seed. The Japanese flowers grown at Swanley appear to be slightly less effective than the Swiss flowers grown at that station, but again this may be true only for these samples and other things being equal, is hardly of practical significance.

Samples of flowers grown at Harpenden, Seale Hayne, in the Scilly Isles, and in France were also tested on young larvae of the Vapourer Moth (*Orgyia antiqua* L.) which are very sensitive to the action of pyrethrum extract (see p. 440). No differences between the four samples were detectable, all the larvae (10 in each test) being almost immediately killed by spraying with concentrations of 1·0, 0·5 and 0·25 per cent.

1927 *harvest*. An opportunity occurred in 1927 to test flowers from a few of the stations shortly after harvesting. On the whole, the results are closely similar to those obtained for the 1926 harvest; the samples of Japanese flowers grown at Wye would appear to be rather more toxic than a sample from the same plot in 1926. The Swanley (Swiss) flowers again show a slightly higher toxicity than the other samples.

Effect of stage of development of the flowers on their toxicity.

It appears to be a common practice amongst certain buyers of pyrethrum flowers to prefer those in which the flower is not completely open, as it is said that adulteration is then more readily detected. Juillet(5) in his monograph (p. 110) adverts to this practice, and states that in commerce great importance is attached to the degree of openness of the flower, the "closed" buds being regarded as much more effective than the "half-closed," which again are supposed to be superior to the open flowers. Juillet considers this view unsound and quotes Faes(2) and Passerini(7) as having demonstrated the "open" flowers to be superior to the "closed" on the one hand, and to the over-blown on the other.

In view of the commercial importance of selecting a right time for harvesting, a number of flower heads at different stages of development were chosen from the same rows of a bed bearing a crop of flowers grown from Swiss seed at Harpenden. The stages at which they were taken are almost identical with those shown in the frontispiece of Juillet's monograph, except that the "closed" buds were beginning to show yellowish green petals.

The stages at which the samples were taken may be described as follows:

1. Closed buds.
2. Buds beginning to show white petals.
3. Half-open flowers.
4. Fully open flowers.
5. Very fully open flowers, *i.e.* had been fully open for some days.
6. Over-blown flowers.

The last two samples were taken from an adjoining bed.

Samples 1–4 were dried at ordinary temperature in the shade; sample 5 was treated in three different ways to be described later; and sample 6 was dried at 50° C. in a drying room. All the samples with the exception of the over-blown flowers were tested on the same day.

The results are given in Table III.

Taking into account the difficulty of evaluating the "moribund," the results obtained clearly indicate that little difference exists between the toxicity of the different samples, weight for weight; the differences observed cannot be regarded as outside the error of the experiment. The views of Juillet, Faes and Passerini are confirmed and there is no warrant for evaluating pyrethrum upon a basis of the degree of opening of the flowers. There are however two points of considerable importance to the grower disclosed by these tests. (1) The crop yields progressively increase in weight with the degree of opening of the flowers; thus the weight of the air-dried flower-heads per hundred is in the following order: Closed, 8·8 gm.; slightly open, 14·0 gm.; half-open, 14·75 gm.; fully open, 20·5 gm.; very fully open, 21·6 gm. By taking the crop in the closed stage, there is a loss in actual yield of insecticide per unit area of nearly 60 per cent., and by taking in the half-closed state, of over 25 per cent., as compared with the yield when the crop is taken when the flowers are fully open[1]. (2) There is little advantage to be obtained in taking the flowers beyond the fully open state as the over-blown flowers

[1] The losses would not be so great under practical conditions since it is not feasible to harvest the crop with all the flowers at precisely the same stage of development.

show no superiority in toxicity; indeed, the sample dealt with by us, shed the disc corollae so readily that no determination of the weight of the dry flower-heads could be made.

Table III.

Effect of degree of development and method of after-treatment of pyrethrum flowers on toxicity to A. rumicis.

(Grown at Harpenden from Swiss seed. Harvest 1927.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Degree of development and method of treatment	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
Buds—closed.	16·8	0·35 and 0·2	—	—	—	100	100
Air-dried at ordinary temperatures		0·1	—	—	20	80	100
		0·05	20	10	50	10	60
		0·025	40	10	20	30	50
Buds beginning to show white petals.	16·8	0·35 and 0·2	—	—	—	100	100
		0·1	—	—	20	80	100
Air-dried at ordinary temperatures		0·05	10	30	40	20	60
		0·025	60	20	10	10	20
Flowers—half-open.	15·1	0·35 and 0·2	—	—	—	100	100
Air-dried at ordinary temperatures		0·1	—	—	50	50	100
		0·05	30	20	50	—	50
		0·025	80	10	—	10	10
Flowers—fully open.	14·5	0·35	—	—	—	100	100
Air-dried at ordinary temperatures		0·2	—	—	20	80	100
		0·1	—	—	40	60	100
		0·05	—	40	20	40	60
		0·025	50	20	20	10	30
Flowers—very fully open.	14·4	0·35 and 0·2	—	—	—	100	100
Air-dried at ordinary temperatures		0·1	—	—	—	100	100
		0·05	40	20	20	20	40
		0·025	30	20	50	—	50 (M
Flowers—very fully open.	14·4	0·35	—	—	—	100	100
Dried at 45–50° C.		0·2	—	—	20	80	100
		0·1	—	—	—	100	100
		0·05	30	20	30	20	50
		0·025	60	30	10	10	20
Flowers—very fully open.	—	0·35	—	—	—	100	100
Soaked without drying in 95 % alcohol		0·2	—	—	70	30	100
		0·1	—	10	60	30	90
		0·05	50	30	20	—	20
Flowers—over-blown. Seeds not ripe.	14·8	0·35 and 0·2	—	—	—	100	100
		0·1	—	10	10	80	90
Dried at 45–50° C.		0·05	10	20	—	70	70
		0·025	50	20	—	30	30

Effect of different methods of drying and after-treatment on the toxicity of the flowers.

In a wet summer there might be some difficulty in drying a crop of pyrethrum in the ordinary way, and a few tests were made to ascertain whether artificial drying could be adopted without loss of toxic properties. Abbott's work(1) indicates that pyrethrum flowers can be subjected to dry heat at 120° C. for 18 hours without any noticeable injury, but temperatures of 130–140° C. for the same length of time destroy the toxic principle. Such high temperatures are not necessary for commercial drying, and these results indicate that there is a considerable margin of safety.

A large sample of fully open flowers was taken on July 19th, 1927, thoroughly mixed and divided into three equal portions which were treated in the following ways:

(1) Dried in a drying room at 40–50° C. for 24 hours and ground before extraction.

(2) Dried at ordinary temperatures, for 14 days and ground before extraction.

(3) Put into 95 per cent. alcohol without drying or grinding.

There was a loss on drying in the first two cases of 72·5–73·3 per cent., the dried samples on heating in an electric oven to 104° C. showing a further loss of 14·4 per cent. on the partially desiccated samples. Sample (3) was filtered through muslin and freed by pressure as far as possible from the extract. The filtrate was then diluted to give a concentration of 10 per cent. calculated on the air-dried sample. Dilutions were prepared from this concentrated extract and their toxicities compared with extracts of similar concentrations prepared from samples (1) and (2). The results of this experiment, which are included in Table III, show that there is little or no difference between the three samples and that artificial drying or even direct pickling in alcohol can be employed after harvesting the crop without any material loss of toxic properties. The slightly lower toxicity values for the wet extracted sample (3) may be significant, but are certainly not of practical importance. They are probably due to incomplete extraction of the unground flower-heads and to the difficulty of freeing them entirely from the last traces of extract. On a large scale this difficulty would be readily overcome. It has, however, yet to be ascertained whether an extract containing so large an amount of water would retain its toxicity over a considerable period of time. It can be deduced from this experiment that difficulty

in air-drying need not be an insuperable objection to the growth and use of pyrethrum in this country.

Toxicity of different parts of the plant.

The results so far discussed have been in all cases concerned with the toxicity of the complete flower-heads, cut off from the stalk just below the receptacle. Comparative tests were also carried out on the stalks, and on the disc and ray corollae separately. These were air-dried in the usual way, and extracted with absolute alcohol. The values obtained are expressed in Table IV. It will be seen that the complete flowers were more than ten times as toxic as the stalks weight for weight; that the ray corollae were not toxic, but that the disc corollae were apparently rather more toxic than the stalks. The corollae are frequently shed in the drying process, but they are obviously of little commercial importance. Large quantities of stalks are however available, and if it is borne in mind that their toxicity is much less than that of the flowers, their use has some justification, provided they are treated by some suitable extraction process.

Table IV.

Toxicities of different parts of pyrethrum plant to A. rumicis.

(Grown at Harpenden from Swiss seed. Harvest 1926.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Part of plant	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
A. Flowers	14·8	0·5 and 0·35	—	—	—	100	100
Tests made Aug. 1926		0·2	—	20	40	40	80
		0·1	30	20	30	20	50
B. Flowers. Same extract		0·35 and 0·2	—	—	—	100	100
as in A.		0·1	10	—	—	90	90
Tests made June 1927		0·05	50	20	10	20	30
C. Flowers		0·5 and 0·35	—	—	—	100	100
Average of tests June–Aug. 1927		0·25 and 0·2	—	—	15	85	100
		0·1	7·5	20	25	47·5	72·5
		0·05	60	17·5	10	12·5	22·5
		0·025	80	10	—	10	10
D. Stalks	12·4	1·0	Not completely toxic				
Tested Aug. 1926							
E. Stalks		5·0	—	—	80	20	100
Tested June 1927		2·5	—	—	30	70	100
		1·0	40	30	20	10	30
F. Disc corollae	15·1	1·0	—	20	20	60	80
G. Ray corollae	16·0	1·0	70	20	—	10	10

Effect of exposure on the toxicity of pyrethrum.

The data set out in Tables II and III were obtained some ten months after the crop had been harvested; the material had been stored in bulk in covered but not air-tight drums and tins, and the samples taken for testing had been stored after grinding in glass-stoppered bottles. It is commonly stated that pyrethrum powder loses its toxicity very readily, and although there is evidence in the literature against this opinion, it seemed advisable to make further experiments on the point.

Abbott(1) has shown that whole and ground flower-heads of pyrethrum are not injured if kept in sealed fruit-jars for 150 weeks; that ground flower-heads in closed glass vessels had lost their toxicity in $5\frac{1}{2}$ years; but that whole flower-heads, kept under similar conditions were practically unhurt, even by such prolonged storage. Further, he found that whole and ground flower-heads could be exposed to the weather in an open dish for 12 weeks, without loss of toxicity, but that an exposure of 21 weeks reduced their potency; on the other hand, whole flower-heads exposed in an open dish in a room, retained their toxicity for 150 weeks, and ground flower-heads for 34 weeks, but the insecticidal value of the latter was reduced by an exposure to similar conditions in 136 weeks. It would appear from these results that pyrethrum under reasonable storage is very much more stable than is commonly supposed; our data tend to substantiate these conclusions.

Certain preliminary figures have been obtained indicating the effect of time on the toxicity of the flowers and stalks kept in stoppered bottles and upon the toxicity of the alcoholic extracts of the flowers. A sample of flowers grown from Swiss seed at Harpenden was harvested in July 1926 and tested in August of that year; the results are given under A (Table IV). Fresh extracts were made on several occasions from June to August in 1927, and the average figures are set out under C. The figures indicate no loss of toxicity on standing, the small differences that appear being almost certainly due to the difficulty of distinguishing with accuracy between the "moribund" and "dead" insects. In 1927 an opportunity occurred of testing the same alcoholic extract as was tested in August 1926, and the figures are set out under B. The difference between the values given under A and B are slight and within the limits of experimental error for this material, and the conclusion may be safely drawn that, between August 1926 and June 1927, the alcoholic extract had shown no loss of toxicity. In order to test the losses of toxicity under varying conditions of exposure a number of experiments

was made during the early part of 1927. A sample of coarsely ground flowers grown from Swiss seed in Harpenden and a sample of stalks grown from Swiss seed were exposed in shallow Petri dishes at Rothamsted in the insectary, a building which, while it afforded protection from

Table V.

Effect of exposure on toxicity of pyrethrum to A. rumicis.

(Grown at Harpenden from Swiss seed.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Treatment and length of exposure	Loss on drying at 104° C. %	Concentration in terms of part of plant gm./100 c.c.	N %	S %	M %	D %	M & D %
First Series							
Flowers—bottled sample	14·8	1·0–0·5	—	—	—	100	100
		0·25	—	—	30	70	100
		0·1	—	40	20	40	60
		0·05	40	40	—	20	20
Flowers—exposed 6 months in shallow dish to open air	14·3	2·0	—	—	—	100	100
		1·0	10	—	20	70	90
		0·5	—	20	10	70	80
		0·25	50	10	30	10	40
Flowers—moistened and exposed 13 days to air saturated with moisture. Much fungus infection	13·5	2·0–1·0	—	—	—	100	100
		0·5	—	10	20	70	90
		0·25	20	20	30	30	60
Flowers—moistened and exposed 2 months to air saturated with moisture (formalin present). No fungus	15·7	2·0–1·0	—	—	—	100	100
		0·5	—	40	20	20	40
		0·25	50	10	10	30	40
Stalks—bottled sample	12·4	5·0	—	—	40	60	100
		2·5	—	30	30	40	70
		1·0	40	10	20	30	50
		0·5	40	10	20	30	50
Stalks—exposed 6 months in shallow dish to open air	13·5	5·0	—	20	80	—	80
		2·5	60	20	20	—	20
Second Series							
Flowers—bottled sample	14·8	0·35	—	—	—	100	100
		0·2	—	—	20	80	100
		0·1	—	30	30	40	70
		0·05	60	30	10	—	10
Flowers—moistened and dried in shade	19·1	0·25	—	—	—	100	100
		0·1	50	—	—	50	50
Flowers—moistened and dried in sun	10·9	0·25	—	—	10	90	100
		0·1	60	10	30	—	30
Flowers—moistened and dried at 40–50° C.	11·4	0·25	—	—	—	100	100
		0·1	10	—	10	80	90
Flowers—kept moist 6 weeks in shade. Much fungus	11·7	0·5	50	20	10	20	30
		0·25	40	10	40	10	50(?)
		0·1	70	10	10	10	20
Flowers—kept moist in open, exposed to sun	12·9	0·5	—	—	30	70	100
		0·25	—	30	30	50	80
		0·1	60	40	—	—	—

rain and snow, could otherwise be regarded as giving conditions equivalent to the open air. The exposure continued for six months from December 8th, 1926 to June 8th, 1927, and the samples were tested on June 9th. The toxicity data and the moisture contents of the samples are set out in Table V together with the values given on this day by the samples stored in stoppered bottles.

The results indicate some loss of toxicity under these drastic conditions of exposure, though this was less than was expected. A sample of Japanese flowers exposed in the same way but not tested so critically showed little or no loss of toxic properties. The sample of stalks exposed in the same way for the same length of time gave values indicating a loss of toxicity.

Another sample of ground Swiss flowers was moistened and exposed in a shallow dish in a glass vessel, used normally as a desiccator, the atmosphere being kept saturated with water vapour. Within a fortnight a considerable growth of fungi was observed and the flowers were therefore air-dried and bottled. This sample showed a loss of toxicity. Another moistened sample was put into the apparatus, but in this case, in order to prevent fungal infection, a little formaldehyde was added to the water standing in the desiccator; after an exposure of two months, the powder was air-dried and tested; it showed some loss of toxicity. Powdered samples were also thoroughly wetted, and then dried in the sun, in the shade, and artificially dried; the loss of toxic properties observed was not great in any of the cases. Two further drastic tests were therefore made; samples in flat dishes were saturated with moisture, and exposed, one in the shade in the insectary, and the other on the roof of the laboratory. The latter sample frequently became flooded and on such occasions was allowed to dry indoors. The sample exposed in the insectary was kept thoroughly moist. The exposure continued for six weeks, by which time a plentiful growth of fungus had taken place on both samples. They were then air-dried and tested; both samples showed loss of toxicity, which in the case of the sample exposed in the insectary was considerable. Under sufficiently drastic conditions, therefore, pyrethrum can be made to lose its toxicity, but it is clear that the commonly expressed objection to its use on the grounds of a supposed rapid loss of toxicity is exaggerated. Obviously, after the crop is gathered, it should be dried at once to an extent which will not allow of fungus growth, but if then stored in a reasonable manner, *e.g.* in well-covered bins, there is no reason to expect any material loss of toxicity for a considerable period of time.

These conclusions are confirmed by some experiments with larvae of the purple thorn moth, the results of which are given in Table VI.

Table VI.

Effect of exposure upon the toxicity of pyrethrum to S. tetralunaria (*larvae*).

(Grown at Harpenden from Swiss seed.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]
(Larvae 25–30 days old.)

Description	Concentration in terms of part of plant gm./100 c.c.	N	S*	M	D
Flowers—kept bottled	2·0	—	2	1	7
	1·0	—	2	1	7
	0·5	—	3	1	7
Flowers—exposed in insectary 8. xii. 26–8. vi. 27	2·0	—	2	1	7
	1·0	—	3	1	7
	0·5	—	3	—	7
Flowers—moistened and exposed to air saturated with moisture. Formalin present	2·0	—	—	3	7
	1·0	—	2	1	7
	0·5	—	1	—	9

* Those recorded under S fed very little and made little or no growth but remained alive.

Toxicity of dried extracts of pyrethrum.

In Tables I–VI concentrations are expressed as percentages of the air-dry flowers or stalks and the results indicate that the actual poison, which is present only to the extent of 0·4 -0·5 per cent. of the flower-heads, must be extremely toxic. McDonnell, Roark and Keenan have shown that petroleum ether, whilst giving a smaller total weight of extracted matter than the other solvents, does extract the whole of the poison. Our experiments substantiate this finding. Five gm. of coarsely ground pyrethrum flowers were extracted in a Soxhlet apparatus successively with petroleum ether (B.P. 40–50° C.) ordinary methylated ether (Sp. Gr. ·720) and absolute alcohol; after the extraction was completed, the solvent was evaporated on the water-bath to a small bulk and then to dryness *in vacuo*. The extracts were weighed, dissolved in absolute alcohol, diluted and tested for their insecticidal values. The petroleum ether extract was completely toxic down to a concentration of 0·01 per cent.

The extractions were done in duplicate, one being tested 14 and 71 days after extracting, having been allowed to stand in the meantime in the form of a concentrated emulsion. The second extract was allowed to stand 85 days in a dry state before testing. Both the petroleum ether extracts which had been allowed to stand for some time were found less

toxic than the sample tested shortly after extraction, indicating some loss of toxicity with time. Neither the ether extract after petroleum ether nor the alcoholic extract after ether showed appreciable toxicities.

Experiments with Caterpillars.

In addition to the experiments with aphides, a limited number of tests were carried out with several species of lepidopterous larvae, the main object being to compare the degree of resistance to pyrethrum shown by different species. Extracts of flowers grown in Harpenden from Swiss seed were used. The technique adopted was similar to that used for aphides; each batch of larvae was sprayed in the standard apparatus and then transferred to separate small cages provided with a supply of fresh food-plant and kept under observation for 8–10 days. The numbers of larvae of two of the species available were rather small, but the results obtained were very consistent and definite and seemed worth putting on record.

Table VII.

Toxicity of pyrethrum flowers to caterpillars of different species.

(Grown at Harpenden from Swiss seed. Harvest 1926.)

[N = not affected. S = slightly affected. M = moribund. D = apparently dead.]

Species and age	Concentration in terms of part of plant gm./100 c.c.	N	S	M	D
S. tetralunaria (Purple thorn moth), 10–15 days old	2·0	—	—	—	10
	1·0	—	2	—	8
	0·5	—	2	—	7
	0·25	—	2	—	8
S. tetralunaria (Purple thorn moth), 25–30 days old	2·0	—	2	1	7
	1·0	—	2	1	7
	0·5	—	3	—	7
**O. antiqua* (Vapourer moth), 3–4 weeks old	1·0	—	—	—	10
	0·5	—	—	—	10
	0·25	—	—	—	10
M. brassicae (Cabbage moth), 10–12 days old	0·5	10	—	—	—
M. brassicae (Cabbage moth), about 15 days old	2·0	7	—	—	3
	1·0	8	—	—	2
	0·5	10	—	—	—
	0·25	10	—	—	—
Pieris brassicae (Cabbage white butterfly), about half-grown	2·0	—	—	—	6
	1·0	—	—	—	6
	0·5	1	3	—	2
	0·25	2	2	—	2

* Similar results were obtained with samples of pyrethrum from Newton Abbot, Isles of Scilly, and from France (p. 431).

Four species were tested—the purple thorn moth (*Selenia tetralunaria* Hufn.), the vapourer moth (*Orgyia antiqua* L.), the cabbage moth (*Barathra* (*Mamestra*) *brassicae* L.), and the large white cabbage butterfly (*Pieris brassicae* L.). The two former were bred from the egg under uniform and protected conditions and were completely free from parasites; the others were wild larvae, collected when quite small. In all cases, the larvae used in the experiments were in a comparatively young stage. The essential details and the results of the tests are given in Table VII.

It will be seen at once that the four species show very different powers of resistance to the toxic action of pyrethrum extracts.

The vapourer moth larvae proved to be very susceptible and all were killed by the spray at all concentrations down to 0·25 per cent.; the toxic action was extremely rapid.

The thorn moth larvae, which were tested at two stages of growth, were a little less easily killed; a small percentage survived at most concentrations, but these were in a semi-paralysed condition, and, though able to feed a little, made little growth.

The white cabbage butterfly larvae were rather older than the other species tested (about half-grown) and proved somewhat more resistant than the vapourer and thorn moths. All were killed however at concentrations of 2 and 1 per cent. At lower concentrations, four out of six survived, though some of these were obviously affected by the poison.

Finally, the cabbage moth larvae, even in a very young stage, showed themselves highly resistant, only two or three out of ten being killed at concentrations of 2 and 1 per cent. Lower concentrations had apparently no effect whatever, the treated larvae feeding and growing normally.

Such marked differences in the susceptibility of different species of insects to the action of pyrethrum have frequently been observed. Juillet, in chapters 14 and 19 of his monograph, discusses the toxic action of pyrethrum, and notes that, although most insects are susceptible, yet there are certain species which possess high powers of resistance, and includes among these *Barathra* (*Mamestra*) *brassicae*, the cabbage moth.

A single experiment with cabbage moth larvae was made to ascertain whether pyrethrum extract would have an action as a stomach poison. Young cabbage plants in pots were sprayed with fluids containing 1 per cent. and 0·5 per cent. of the flowers with 0·25 per cent. soft soap, and after being allowed to dry, young larvae were caged on the plants. The larvae were apparently entirely unaffected; they fed on the sprayed foliage, grew normally, and were indistinguishable from the control larvae

feeding on plants sprayed with 0·25 per cent. of soft soap only, or unsprayed. There was no evidence that the pyrethrum extract had any action as a stomach poison or as a repellent to these insects.

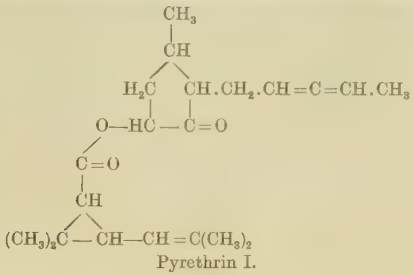
Discussion.

The data given in the experimental part of this paper confirm the results of other workers as to the high insecticidal value of pyrethrum (*C. cinerariaefolium*), and show that this plant can be successfully grown in England and harvested without loss of toxicity under our climatic conditions. The material raised here has about the same insecticidal value as samples received from abroad. The results also indicate that the crop can be air-dried at ordinary temperatures, or artificially dried if necessary, without damage, and that the air-dried flowers may be stored in covered vessels for considerable periods with little or no deterioration. Prolonged exposure to wet conditions may however lead to some loss of toxicity. We find no justification, so far as the toxicity of the product is concerned, for the opinion that it is better to harvest the crop when the flowers are "half-closed" than when they are fully open. We have not however dealt with the bearing of this practice on the detection of subsequent adulteration of the dried flowers.

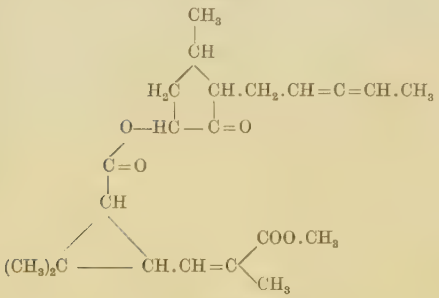
McDonnell, Roark and Keenan (6) deal with this phase of the pyrethrum question at some length, and they have suggested microscopical and chemical methods for the detection of adulteration. Their chemical methods do not, however, depend upon the isolation or determination of any constituent which is directly colligative with the toxic principle of the plant. This indeed was impossible until the active principle was discovered and its constitution determined. A satisfactory method of comparing the toxicities of different samples of pyrethrum either directly by biological methods or by estimation of the toxic constituents would be of considerable economic importance for the selection of varieties with higher poison contents and for the evaluation of individual samples.

The biological method used in the work described in this paper has proved admirably adapted for the purpose of evaluating the samples we have had to test, and has indeed been used for the detection of pyrethrum extracts in insecticidal material of unknown composition. The detection of major differences in toxicity between different samples has been aimed at in the present work, but, by using larger numbers of insects and concentrations nearer together, smaller differences in toxicity could undoubtedly be detected and estimated by this method.

In recent years, a considerable amount of research has been carried out on the chemical composition of the active principles of pyrethrum. In 1909, Fujitani (4) isolated a thick yellow syrup, highly toxic to insects, to which the name Pyrethron was given, and this material was further studied by Yamamoto (12); we are however chiefly indebted to the classical researches of Staudinger and Ruzicka (9) for our knowledge of the constitution of the active principles. These investigators isolated in a pure state two chemical compounds highly poisonous to insects, named by them Pyrethrin I and II, and the structure of both compounds was elucidated. The constitutional formulae ascribed to them are as follows:



Pyrethrin I.



Pyrethrin II.

Both of these bodies are excessively toxic to insects. Pyrethrin I is the more active and, according to these authors, killed cockroaches at a dilution of 1 in 10,000 in 10 to 20 min.; pyrethrin II was less toxic and required 20 to 40 min.

Staudinger and Ruzicka ascribe the high toxicity of the pyrethrins to their peculiar structure as esters of trimethylene carboxylic acids with unsaturated side chains and a cyclopentalone derivative with an unsaturated side chain. Small changes in either the alcoholic or acidic portions of the molecule caused a profound reduction in toxic properties. They found between 0·2 and 0·3 per cent. of pyrethrin in the flower-heads

of the samples they were dealing with. The isolation of these compounds in a pure state is complicated and depends on the preparation of their semi-carbazones.

It is evident from the work of these authors that any chemical method for the quantitative evaluation of pyrethrum which aims at a separation of the semi-carbazones of the actual insecticidal substances present is likely to be difficult and unsuitable for routine work. They themselves suggest that pyrethrin might be determined by isolating the semi-carbazones of the alcohol pyrethrolone but the process is not a simple one.

Recently, Staudinger and Harder(8) have published an account of two somewhat simpler methods of estimating pyrethrin I and II, and found that the pyrethrin content of a number of samples of pyrethrum flowers ranged from 0·4 to 0·6 per cent., a higher figure than that obtained by the older method. The pyrethrin content of the stalks varied between 0·04 and 0·1 per cent. The data at present available indicate that the difference in the pyrethrin content of flowers and stalks is of the same order as the difference in the toxicity of flowers and stalks as determined by our biological method. Further, Staudinger and Harder found only insignificant differences between the pyrethrin contents of closed, half-open and fully open flowers, which is again in agreement with the results of our toxicity experiments. We hope, at a later date, to discuss the chemical method of Staudinger and Harder in greater detail and to compare the results further with those obtainable by the biological method.

It would be of interest to ascertain the pyrethrin content of samples of genuine pyrethrum in which toxicity had been lost, as *e.g.* by prolonged exposure to wet conditions. The most probable explanation of such loss of toxicity is that it is due to hydrolysis of the Pyrèthrin. The losses of toxicity in alcoholic-soap extracts of pyrethrum (Savon-pyrèthre) are thus explained by Staudinger and Harder. Another source of loss, however, pointed out by these investigators, is one due to alcohol-radical exchanges (ester exchange); methyl alcohol gives rise readily to the methyl esters of the acids and free pyrethrolone which are not toxic, and though ethyl alcohol apparently reacts more slowly, it is nevertheless not considered by them to be suitable for technical extracts and they propose for the purpose indifferent organic solvents such as benzene, petroleum ether, trichlorethylene and acetone. Harder has shown that extracts of this type can be emulsified by turkey-red oil and are quite suitable for use in insect control. So far, in our experiments, extracts of pyrethrum containing 14 to 15 per cent. of moisture made with absolute

ethyl alcohol have retained their toxicity for many months; we have not, however, determined the length of time that the toxicities of extracts made with commercial spirit would be maintained.

Summary.

1. The toxicity to *Aphis rumicis* L. and to certain caterpillars of spray fluids prepared from samples of pyrethrum (*Chrysanthemum cinerariaefolium*) grown in England from Swiss and Japanese seed have been quantitatively determined.

2. Pyrethrum flowers, grown in six different localities, showed only slight differences, and, for practical purposes, all the samples had approximately the same toxicity. They did not differ in this respect significantly from a sample grown on the continent.

3. The toxicities of extracts of equal weights of pyrethrum flowers tested at different stages of development differed very little.

4. Artificial drying of the flowers had no significant effect on the toxic properties.

5. The flowers were about ten times as toxic as the stalks, weight for weight.

6. Prolonged exposure of pyrethrum to wet conditions led to some loss of toxicity, but contrary to the usual opinion, if stored in a reasonable manner, it remained for long periods without deterioration.

7. Caterpillars of different species showed marked differences in susceptibility to the action of pyrethrum.

8. The biological method employed has proved suitable for evaluating samples of pyrethrum.

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THE COMMON GREEN CAPSID BUG (*LYGUS PABULINUS*)

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(With Plates XXI and XXII, and 8 Text-figures.)

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Introduction.

Numerous complaints of the serious damage done to currants and other plants during 1925 were received from various parts of the Eastern Counties. An investigation of the damage showed that in most cases the Capsid bug *Lygus pabulinus* was the culprit although in some cases the damage to currants was caused by *Plesiocoris rugicollis*.

Mr H. Goude, the Horticultural Superintendent for Norfolk, regarded this damage as a very serious matter for the black currant growers in Norfolk and he requested that a detailed study of the trouble should be undertaken.

As *L. pabulinus* was found to be the chief culprit, a detailed study of

its life-history was undertaken in the hope of finding data likely to be of importance in the economic control of this pest.

For some years damage by this pest to currants, gooseberries, strawberries and potatoes has been noticed in many districts in East Anglia.

An important observation of biological interest and possibly of very great economic importance is the finding of *L. pabulinus* causing very similar damage to apple leaves as does *Plesiocoris rugicollis* in several districts in Cambridgeshire, the Isle of Ely, Norfolk and Suffolk. Previous observations on the apple capsids in these districts from 1915 to the present failed to reveal the presence of this bug on apple trees until 1926. In 1917 a very large number of the capsids on apple trees were examined by Petherbridge and Husain (9) without revealing the presence of this bug.

Smith (18) has reared this species on apple from the first stage to the adult stage and says "it would be well to recognise the possibility of capsids such as *Lygus and its allies* suddenly developing a taste for fruitarian diet."

On May 27th, 1919, *L. pabulinus* (chiefly 4th stage) was found marking the leaves of young pear trees at Walton near Wisbech and also the neighbouring gooseberries and strawberries. The pear trees were removed soon afterwards and no further damage of pear leaves was noticed until 1925.

Mr J. C. F. Fryer (*in litt.*) records this pest as damaging pear fruit in 1914 at Salkeld, Penrith.

K. M. Smith (17) also recorded this pest as causing black spotting of the fruit and leaf of the pear.

In 1917 most of the typical capsid damage on currants in the Cambridge and Wisbech districts was caused by *P. rugicollis*, but in 1925 and 1926 much more damage was found to be caused by *L. pabulinus*, although in some gardens *P. rugicollis* was still doing most of the damage.

We have found *P. rugicollis* marking only apples, currants, willows, and a single specimen marking a strawberry plant growing under an apple tree which was also attacked, whereas we have found *L. pabulinus* marking a large number of other plants.

P. rugicollis and *L. pabulinus* are the only capsids that we have found on fruit trees causing the characteristic brown capsid marking.

Smith (17) has shown that in the case of these two capsids the cells of host plant surrounding the puncture are killed by the presence of a toxic substance in the saliva which is injected into the plants.

It may here be mentioned that in contradistinction to *P. rugicollis* there are two generations each year in the case of *L. pabulinus*.

Methods.

Observations were started in fruit plantations near Wisbech and King's Lynn which were known to be badly attacked in 1925, and also near Cambridge. Later on bad attacks were found at Bluntisham and Earith in Hunts.

Shoots from infested plants were examined and eggs found. A number of these shoots were struck in pots and kept out of doors to get some idea of the time of hatching. Others were kept in the laboratory to hasten the hatching period.

Plantations were examined periodically to enable us to observe the habits of the various bugs. Many bugs were also kept in sleeves and cages and from our observations in the field and from these we were able to decide the duration of the various instars.

Sleeves gave much better results than cages, as in the latter the bugs are difficult to keep alive.

Adult (including genitalia).

Bright green, shining, elongate, oval, punctured, clothed with *dusky* hairs; second segment of antennae green at base, testaceous in middle, black at tip; third and fourth segments black. Pronotum with a narrow raised collar, sides nearly straight, base rounded, anterior callosities moderately developed; scutellum with transverse wrinkles; elytra elongate, their sides slightly curved, membrane irridescent with a longitudinal cloud below the apex of the cells, veins pale green; femora elongate, tibiae with *moderately developed* spines.

Lygus pabulinus.

Male genitalia (Fig. 1). Basal plates separate, thin and frequently difficult to distinguish. Two of the vesical diverticula heavily chitinised and pointed, the rest covered with minute tubercles.

Vesical spine present on left side, originating from the proximal region of the vesica.

Ejaculatory duct widened slightly at base of vesica. Rim of gonopore thick and heavily chitinised.

Paramere asymmetrical, the right one being much expanded at the base.

Female genetalia (Fig. 2). The ovipositor is composed of two pairs of appendages apparently belonging to the eighth and ninth abdominal segments respectively. When at rest it lies in a groove on the ninth abdominal sternite.

The appendages of the eighth segment are two long blade-like structures each connected at the base to a cross-shaped chitinous strut. The

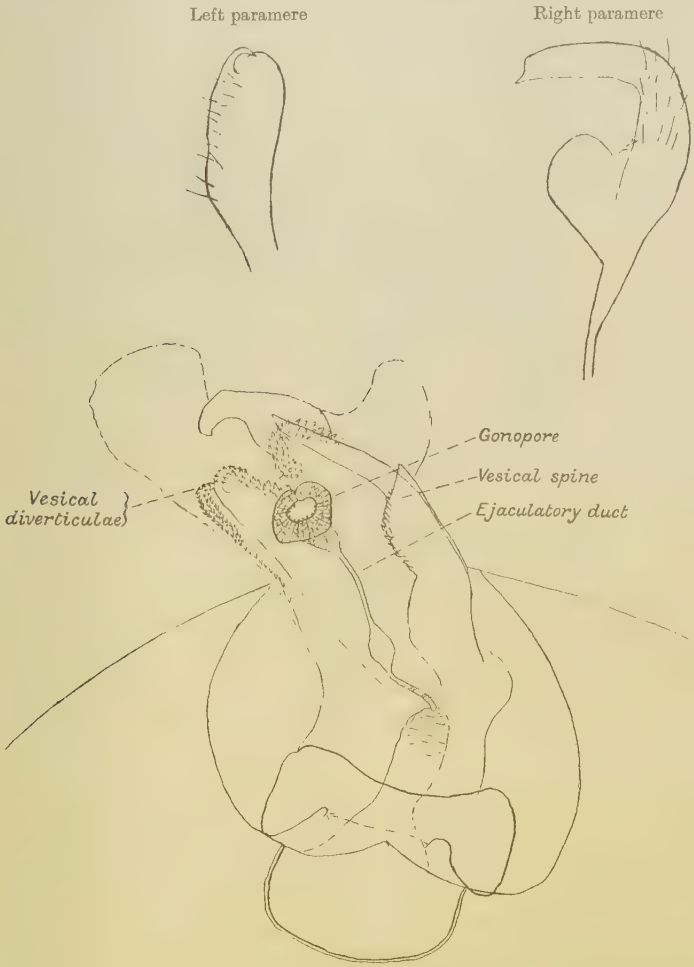


Fig. 1. *Lygus pabulinus.* Male genitalia.

bases of these appendages run forward to the base of the groove where they are sharply bent and thus come to lie together in the groove pointing posteriorly. They are enclosed by the appendages of the ninth segment

which are fused together basally at their dorsal edges and thus form the sheath. These outer lobes are about one-third of the total length of the inner lobes as they lack the long bases characteristic of the latter. The outer lobes each possess a longitudinal ridge on the inner surface, this ridge articulating with a groove on the outer surface of the inner lobes, which are thus capable of sliding longitudinally within the sheath, but all lateral movement relative to the sheath is prevented.

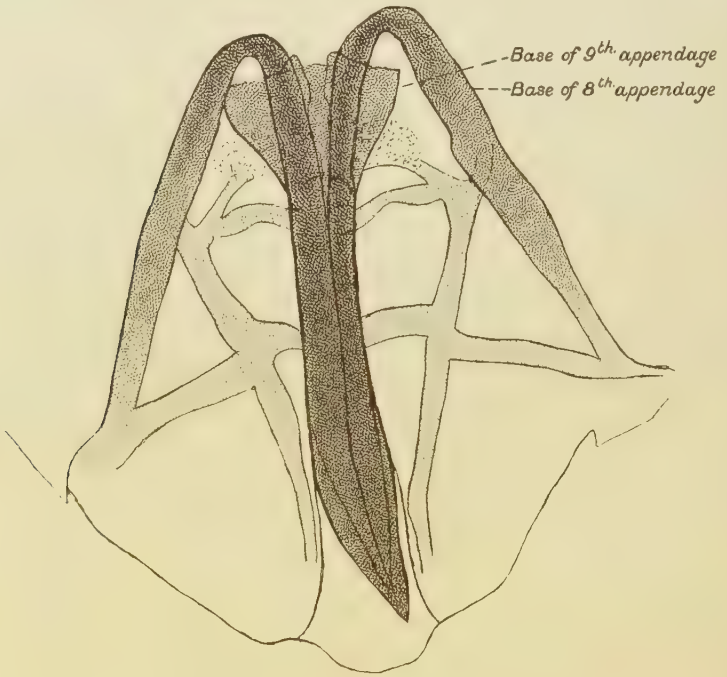


Fig. 2. *Lygus pabulinus.* Female genitalia.

Both pairs of lobes are provided with strong cutting teeth at the apex. The serrations of the inner lobes are blunt and directed backwards, *i.e.* towards base of the ovipositor, and are similar on both sides of the blade. The serrations of the outer lobes are also blunt but point forward, *i.e.* towards apex of the ovipositor. They are regular on the anterior margin but are very irregular on the posterior (dorsal) edge.

Description and Measurement of Egg.

The egg (see Fig. 10) is cream coloured, and the surface is smooth and glistening. The cap is a pale greenish yellow and in the autumn often shows up conspicuously against the stem of the host plant. The cap of the winter egg however may become stained and so matches the bark as closely as that of *P. rugicollis*. On the average of measurements the egg of *L. pabulinus* is shorter and wider than that of *P. rugicollis*, the figures being as follows:

	Length	Greatest width
Plesiocoris rugicollis	1·4 mm.	0·3 mm.
Lygus pabulinus	1·29 mm.	0·37 mm.

The upper part of the egg of *P. rugicollis* is much narrower than that of *L. pabulinus* and the differences are well seen in Fig. 10.

Egg-laying.

During the second week in September 1926 several females were carefully watched but were not observed to lay eggs until September 14th. On that date a single female was watched near the buds of the tips of black currant shoots from 11 a.m. until 12.45 p.m. and from 2.15 p.m. until 3.10 p.m. without laying eggs.

She then walked down the shoot for a distance of about a foot and arrived at a position 2 in. below the top of the second year wood. During the walk down the shoot she appeared to be testing the bark with her proboscis. Twice her ovipositor was inserted and quickly withdrawn. After each insertion the bug moved forward. At the third insertion the ovipositor was inserted almost vertically and then half of it removed and after nearly a minute the egg passed down the ovipositor which was re-inserted. After the egg was laid the bug took nearly three minutes to remove its ovipositor and did so by moving her body from side to side.

The whole process lasted four minutes.

Whilst ovipositing the female remained facing the base of the shoot as was to be expected from the structure of ovipositor which is concave on the posterior side when depositing (*i.e.* dorsal side when at rest) and the position of the eggs previously found in the shoots. Shortly after ovipositing the bug moved to a leaf and then on to another shoot where she stayed for about a quarter of an hour. Another insect then flew past and the bug flew away in the opposite direction to some nettles and was lost sight of. A careful search on the shoot on which the egg was laid revealed the presence of another egg about $1\frac{1}{2}$ in. above the first one.

During the egg-laying period the adults do not appear to feed very much on the leaves and shoots of currants and gooseberries and throughout this period they were always more abundant on the herbaceous plants near by. In some nurseries they fed readily on apples, plums, cherry stocks and roses.

Position of the Eggs.

The over-wintering eggs usually occur singly, but occasionally two or three may be found close together.

They are usually to be found in the present year's shoots, but in the case of currants they were often found in the two-year-old stem.

The eggs are inserted in the stem, usually, with the cap more or less on a level with the surface of the bark. In the case of red currants occasionally some were found not completely inserted, but with one-third or more of their length outside the bark. This is more usual in one-year-old shoots. Preference is given to lenticels, but quite a number are laid elsewhere.

From a superficial examination they are more easily found in the autumn because of the colour contrast in the cap and its surroundings. Sometimes a slightly raised patch caused by the body of the egg can be detected.

If the bark is peeled carefully, the eggs may be seen on its inner surface, but if only the outer cork layer is peeled the eggs remain *in situ*.

It is usually placed so that the longer axis of the cap is parallel with that of the stem, the body of the egg projecting into the tissue sometimes almost radially and sometimes tangentially but with the distal end lying towards the tip of the twig. This is the natural consequence of the position taken up by the female when laying, lengthways on the twig and head downwards, in this way maximum grip and easiest possible insertion being obtained, exactly the same method being adopted by *P. rugicollis* (9).

The body of the egg lies embedded in the phloem and occasionally penetrate a short distance into the xylem. Usually the plant tissue around it remains healthy save near the point of insertion; quite a number of those found were dead and flattened, for no apparent reason, the tissue surrounding them being also dead.

The eggs laid by the spring brood are inserted in the same way in the stems of herbaceous plants but apparently with much less exactness, sometimes with half their length projecting above the surface of the stem while very often eggs lie very obliquely. Owing to the absence of bark

and the fact that they are not so deeply embedded, those laid in herbaceous plants are very easily seen. Most of these summer eggs are laid in the upper part of the plant chosen.

Time of Egg-laying and Hatching.

The first summer egg was found on June 21st, 1926, and the period of greatest activity in egg-laying lasted about 26 days, from June 21st till July 17th, although no doubt a few are laid after this date, as adults containing eggs were found after July 17th and linger on till the beginning of August. The incubation period is from 19–21 days and hatching took place over a period of approximately 16 days, *i.e.* July 13th–28th.

The second generation lay their eggs in September and early October. These remain throughout the winter and commence hatching early in April. In 1926 the eggs at Wisbech were hatching over a period of about 14–16 days, *i.e.* April 3rd–19th, but in 1928 took much longer.

Number of Eggs.

It is difficult to ascertain exactly the number of eggs laid by a female during her lifetime. Egg-laying, in the case of the summer generation continues for about 3–4 weeks, 26 days being the figure given by our field observations. The rate of egg-laying by the bugs kept in cages was very erratic. The most usual rate seemed to be one egg in about 12–15 hours but in one case six bugs laid 40 eggs on strawberry in 36 hours.

And in another case 2 females laid 10 eggs in 22 hours.

Dissection of bugs late in the season which had apparently died a natural death revealed in several cases three or four well-formed eggs in the ovarioles and several probably capable of ripening. Dissection at the commencement of the laying season showed 16-20 well-formed eggs in the ovarioles with many as yet undeveloped oocytes in the germaria.

In the autumn brood the same thing was found; a female dissected on September 13th containing 56 eggs and another dissected on October 6th containing 10 well-formed eggs and at least one other forming.

Description of the Instars.

Instar I (Fig. 3). Length 1·22–1·54 mm.

Small, slender, fragile, semi-transparent, shining. Very pale on hatching, later turning yellowish green. Smoky patches on head and thorax less pronounced than in *P. rugicollis*. Mid dorsal pale line bifurcating on head at level of eyes.

Head. Relatively large. Row of 10 longish hairs on vertex, and some on sides and front of head. Eyes deep carmine, nearly touching the pronotum.

Abdomen. Pyriform. Longer than broad. Row of short dusky hairs on each segment. Greatest breadth at segments 2–4. Small median

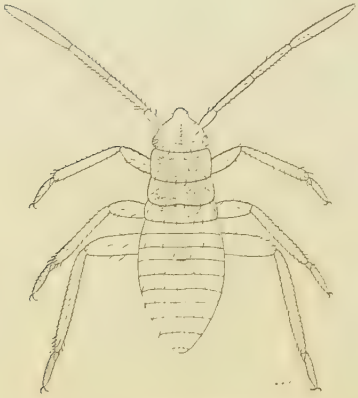


Fig. 3. *Lygus pabulinus.* Instar I.



Fig. 5. *Lygus pabulinus.* Instar III.

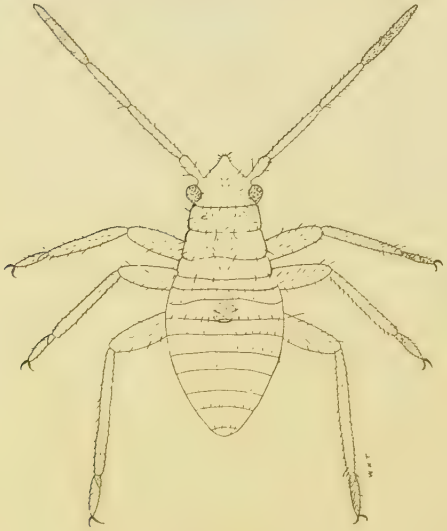


Fig. 4. *Lygus pabulinus.* Instar II.

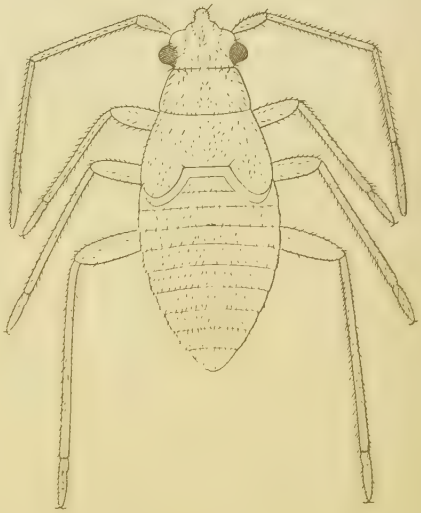


Fig. 6. *Lygus pabulinus.* Instar IV.

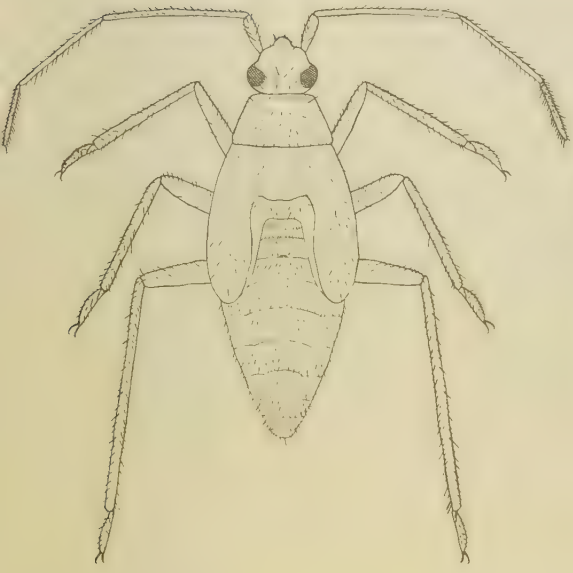


Fig. 7. *Lygus pabulinus.* Instar V.

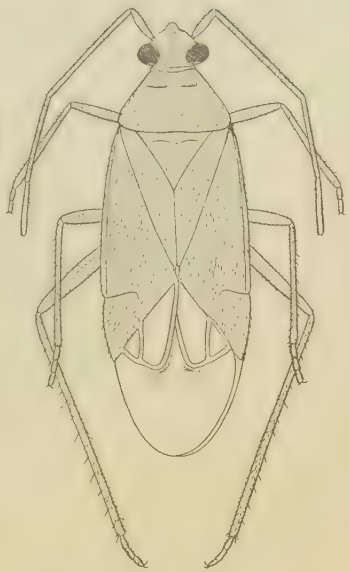


Fig. 8. *Lygus pabulinus.* Adult.

smoky patch on first segment. Dorsal abdominal gland showing as a circular orange yellow patch on segment 3; the opening with dark brown lips between segments 3 and 4.

Antennae. Almost as long as the body, at first almost colourless, later yellowish. Terminal segment dusky orange, thickly clothed with pale hairs, about one and a half times the length of the third segment.

Legs. Long, very pale, especially the posterior ones; tarsi 2-segmented, dusky, almost black at tip, with black claws.

Proboscis. Pale yellow with dark tip, covered with short pale hairs; reaching to the posterior coxae.

Instar II (Fig. 4). Length 1·84–2·09 mm.

Whole pale shining green with a tinge of yellow, later becoming brighter green.

Head. Large, no groove or bifurcating line present. Number of facets increased and widely separated, the area between the facets pale yellow.

Thorax. Two callosities present on pronotum. Mesonotum much longer than in Instar I.

Abdomen. Pyriform; longer than broad. Greatest width at third

segment. Dorsal gland visible as pale yellow spot, lips slightly marked dark brown. A depression is visible near the edge of each segment giving a flat margin to the otherwise arched abdomen. Hairs on terminal abdominal segments longer.

Antennae. Second segment longer than third. Basal segment pale yellowish green; second and third dusky yellow; fourth light brown.

Legs. Pale smoky yellow, tarsi smoky brown.

Rostrum. Pale yellow, almost black at tip.

Instar III (Fig. 5). Length 2·27–2·67 mm.

Colour vivid green.

Characteristic peculiarities. Wing pads now visible, being marked off by a diagonal furrow, the pads being markedly paler than the rest of the thorax. "Margin" to abdomen well-marked. Gland rather faint. Second segment of antenna longer than segments 3 or 4 which are equal. Paired callosities present on both pro- and mesonotum.

Instar IV (Fig. 6). Length 2·83–3·36 mm.

Colour vivid green. Further increase in relative size of second antennal segment; third segment now longer than the fourth. Wing pads just reaching second abdominal segment.

Instar V (Fig. 7). Length 4·15–4·30 mm.

Colour vivid green. Third segment on antenna much longer than fourth; further increase in size of second segment. Wing pads extending very nearly to posterior border of fourth abdominal segment. External genetalia become visible.

Comparison of Early Stages of *P. rugicollis* and *L. pabulinus*.

In all stages the antennae and legs are relatively longer in *L. pabulinus*. Differences other than those based on actual measurements (for which see Table I) must be used with caution owing to the individual variation and the great differences in appearance due to whether the bug is starved or fully fed. By far the most reliable measurements are those of the antennae and of the tibia of the metathoracic leg.

In all stages the terminal joint of the antenna is redder in *P. rugicollis*.

		P. rugicollis	*L. pabulinus*
I.	Total length	1·1–1·4 mm.	1·22–1·54 mm.
	Tibia, 3rd leg	Length 0·45 mm.	Length 0·66 mm.
	Antennae	Length 0·82 mm.	Length 1·16 mm.
		Yellowish green, later dusky. Terminal segment reddish brown	At first almost colourless, later yellowish. Terminal segment dusky orange
	Rostrum	Reaching to middle coxae	Reaching to posterior coxae

		P. rugicollis	*L. pabulinus*
II.	Total length	1·5–2·2 mm.	1·84–2·09 mm.
	Tibia, 3rd leg	Length 0·69 mm.	Length 0·92 mm.
	Antennae	Length 1·05 mm.	Length 1·68 mm.
	Colour	Pale yellowish green	Pale bright green
	Y-line on head	Present	Absent
	Lips of abdominal gland	Well marked	Faintly marked
III.	Total length	2·1–2·8 mm.	2·27–2·67 mm.
	Tibia, 3rd leg	Length 0·98 mm.	Length 1·17 mm.
	Antennae	Length 1·61 mm.	Length 2·10 mm.
	Colour	Dull greenish yellow	Vivid shining green
IV.	Total length	2·7–3·4 mm.	2·83–3·36 mm.
	Tibia, 3rd leg	Length 1·5 mm.	Length 1·77 mm.
	Antennae	Length 2·10 mm.	Length 2·94 mm.
	Colour	As Instar III	As Instar III
V.	Total length	3·6–4·27 mm.	4·15–4·30 mm.
	Tibia, 3rd leg	Length 1·95 mm.	Length 2·43 mm.
	Antennae	Length 3·01 mm.	Length 4·03 mm.
	Colour	As previous instar	As previous instar

Table I.

L. pabulinus. *Measurements in millimetres.*

							Adult	
		I	II	III	IV	V	♂	♀
Head	Length	·27	·37	·44	·53	·71	·66	·76
	Width	·37	·56	·63	·75	·85	·83	·93
Prothorax	Length	·20	·25	·31	·40	·58	—	—
	Width	·41	·59	·64	·83	1·1	Apex ·61 / Base 1·56	·66 / 1·79
Mesothorax	Length	·14	·24	·27	·42	·56	—	—
	Width	·47	·73	·83	1·03	1·70	—	—
Metathorax	Length	·10	·12	·15	·19	·19	—	—
	Width	·49	·83	·88	—	—	—	—
Abdomen	Length	·51–·83	·86–1·1	1·1–1·5	1·29–1·82	2·11–2·26	—	—
	Width	·51	·85	1·02	1·29	1·55	—	—
Total length		1·22–1·54	1·84–2·09	2·27–2·67	2·83–3·36	4·15–4·30	4·25–5·27	5·59–6·46
Antennae	1	·14	·19	·24	·34	·44	·58	·61
	2	·29	·49	·70	1·04	1·50	1·70	1·85
	3	·27	·44	·58	·85	1·13	1·12	1·34
	4	·46	·56	·62	·71	·81	·82	·95
Total length		1·16	1·68	2·10	2·94	4·03	4·22	4·75
Leg I	Femur	·34	·54	·61	·85	1·04	1·16	1·41
	Tibia	·43	·59	·73	·97	1·39	1·53	1·73
	Tarsus	·22	·27	·31	·37	·48	·54	·59
Leg II	Femur	·39	·56	·73	·93	1·03	1·48	1·58
	Tibia	·49	·68	·85	1·22	1·65	1·90	1·99
	Tarsus	·24	·29	·31	·39	·47	·56	·61
Leg III	Femur	·47	·66	·76	1·16	1·5	1·84	1·97
	Tibia	·66	·92	1·17	1·77	2·43	2·80	3·09
	Tarsus	·27	·31	·37	·48	·58	·61	·70
Rostrum		·78	1·02	1·16	1·44	1·73	1·87	1·99

Lygus pabulinus. *First generation.*

Stages	Dates of hatchings and of the various instars	
	1926	1927
Hatching	April 4th	April 20th
Instar I	Present from April 4th–29th	Present from April 20th–May 15th
Instar II	Present from April 14th–May 13th	Present from April 29th–May 23rd
Instar III	Present from April 26th–May 24th	Present from May 7th–May 31st
Instar IV	Present from May 6th–June 6th	Present from May 16th–June 14th
Instar V	Present from May 18th–June 20th	Present from May 23rd–June 25th
Adult	Present from June 2nd–Aug. 3rd	Present from June 4th–Aug. 2nd
Eggs found	June 21st	

N.B. These figures cannot be compared with the figures given by Petherbridge and Husain for *P. rugicollis* as their observations refer to the year 1917 when hatching started very late, namely on May 5th, whereas in 1926 *Plesiocoris* was first found on April 3rd, and in 1927 on April 8th. In these years *P. rugicollis* became adult on June 1st and 2nd.

Second generation.

Stages	Dates of hatchings and of the various instars	
	1926	1927
Instar I	Present from July 13th–July 28th	First found July 21st
Adult	Present from Aug. 9th–Oct. 8th	First found about Aug. 15th (exact date not recorded) Last found Oct. 20th

Habits.

One important characteristic of this insect is the possession of two generations per annum and in this it differs from *P. rugicollis* and other capsid bugs found on apples. Another important feature is the migration of a large percentage or all of the larvae of the first generation from the hosts on which they are hatched (such as currants, gooseberries, apples) to herbaceous plants (such as strawberries, potatoes, bindweed, etc.) on some of which this generation lays the majority of its eggs. The second generation on reaching the adult stage return once more to the woody host plants to lay the winter eggs.

The nymphs (especially in the later stages) fall readily from the plants or bushes and usually spread from plant to plant before reaching the adult stage. This characteristic should be remembered in connection with the control of *L. pabulinus*. *P. rugicollis* does not fall from the trees as readily as does *this pest.*

The adults fly readily when disturbed, and even when not disturbed they often fly from plant to plant in sunny weather. In this they differ from *P. rugicollis* which is rarely seen on the wing. We have evidence that *L. pabulinus* flies readily from one field to the next.

In the plantations visited during April 1926, large numbers of larvae were found feeding on the leaves of both black and red currants. Gooseberries were fairly freely attacked and in three orchards a few larvae were found on young apple trees. This last record is of particular interest as *L. pabulinus* was not found on apple trees among the large number of capsids examined in this district in 1913 by Fryer(8) and by Petherbridge and Husain(9). It seems fairly certain therefore that this habit is a comparatively new one, at any rate, in this part of England, although it has been recorded on the Continent.

The following observation may have some bearing on this change of habit.

In August 1926 young maiden apple trees in a small nursery bed were found entwined with bindweed and the latter was badly marked by *L. pabulinus*, fifth stage larvae of which were still feeding on it. In 1927 these apple trees were attacked by this pest although there was no sign of attack in 1926.

In 1927 this bug was found damaging apples and stocks in a number of nurseries in the eastern and south-eastern counties, and was also found in several orchards on young apple trees.

One interesting case of the introduction of this pest on nursery stock came to our notice. Ten thousand one-year-old crab apple stocks were imported from Holland in December 1926 into a nursery in Cambridgeshire. When this nursery was examined on May 17th, 1927, every one of these was attacked by *L. pabulinus*. No sign of damage was found on neighbouring currants or other worked apples or weeds. Moreover, an examination of these stocks revealed the presence of several eggs agreeing in measurements with those of *L. pabulinus*. These eggs and the presence of the young bugs on the imported stock only[1] provide ample evidence that the eggs were present in the stocks when they were imported in December.

The early attack on currants was very irregular, some bushes being very badly infested and others comparatively free. It is difficult to assign any reason for this; in some cases the bushes most heavily attacked were those nearest the hedge, where the wealth of herbaceous plants would afford ample food for the summer generation, but this did not hold good elsewhere. Red currants seemed to suffer more than black currants, although both were badly infested. Some varieties of red currants were more severely damaged than others in some gardens, but

[1] We were extremely fortunate in our evidence as we examined very few nurseries without finding this pest.

we were unable to obtain definite information as to the names of the varieties. For some time after the appearance of the bugs on currants none whatever could be found by sweeping strawberries, nettle and other herbaceous plants in the vicinity of infected bushes and examination of the plants revealed no signs of the typical injury.

The Aphis (*Amphorophora cosmopolitanus*, Mason) attack in 1926 was very severe and it was noticed, both near Wisbech and King's Lynn, that those bushes which suffered much from capsid were usually less heavily infested by Aphis, and *vice versa*. This is interesting in view of the suggestion that *L. pabulinus* is to some extent aphidivorous.

Anthocoris sylvestris was common on the bushes, but no relationship with the numbers of *L. pabulinus* was noticed.

The first sign of migration was observed on April 28th, 1926, at Bluntisham, one or two strawberry plants, growing close to infected bushes, showing signs of damage and second and third stage bugs were found on them. By May 6th a few larvae could be found on nearly all the strawberry plants, most of them then being in the third instar. In 1927 similar migration was first observed on May 1st, at Wisbech, the attacked plants again being strawberries.

When no strawberries are present in the plantations migration takes place to various herbaceous plants such as potatoes, mint, *Urtica urens* and *dioica*, *Ranunculus repens*, *Senecio vulgaris*, *Convolvulus arvensis*, *Lamium album*, and other host plants (see Host Plants).

When plantations are kept clean a larger percentage of the bugs are found on the currants and gooseberries.

This tendency to go to herbaceous plants may originate with individuals falling off the bushes, and being unable to return, are forced to make the best of whatever food they may find at a lower level. It is, however, quite evident that there is some other factor at work, as later instar bugs in cages show a decided preference for herbaceous plants, whereas the bugs in the earlier instars show a preference for their woody hosts, as seen in the following results from cages in which each host plant was easily accessible.

On May 5th, 1927, when migration had hardly begun, 21 larvae were placed in a cage containing sprays of black currant, dead nettle, stinging nettle and *Sysimbrium alliaria* (hedge garlic). Before starting the experiment all the larvae were shaken off the main sprig of currant on to the other food plants.

After 18 hours a count was made of the bugs on the various food plants, the results being as follows:

	Cage *A*	Cage *B*
Black currant	11	11
Dead nettle	5	3
Stinging nettle	4	2
S. alliaria	1	1

On May 5th, 52 bugs were taken in the same way, giving the following results:

Black currant	20
Apple	9
Dead nettle	15
Stinging nettle	4
Groundsel	3
S. alliaria	1

Note. In this experiment the herbage of the four herbaceous plants was markedly in excess of that of the two woody plants.

At a later date (May 20th, 1927), when migration was in full swing, further experiments gave results strongly in contrast to those obtained previously.

Cage *A*		Cage *B*	
3 sprays currant	7	2 sprays currant	5
2 sprays dead nettle	9	2 sprays dead nettle	13
2 sprays groundsel	8	1 spray stinging nettle	0
1 spray *S. alliaria*	2		
Totals	26		18

In both these experiments the currant leaf was much in excess of that of any other *one* plant; in fact there was a slight excess of currant over all other plants together.

Experiments at a corresponding time in 1926 gave similar results.

May 12th, 1926.

Cage I		Cage II	
Currant	1	Currant	3
Sysimbrium alliaria	1	*Urtica dioica*	6
Lamium album	8	*Lamium album*	5
Potato	15	Potato	6
Totals	25		20

A summary of these experiments over the two years will show the nature of the results more clearly.

Before commencement of Migration.

Bugs choosing winter host plant (*i.e.* currant and apple)	50	56 %
Bugs choosing herbaceous plants (*i.e.* potato, dead nettle, stinging nettle, groundsel and hedge garlic)	39	44 %

After commencement of Migration.

Bugs choosing winter host plant	16	20 %
Bugs choosing herbaceous plants	63	80 %

In addition to the migration from woody plants to herbaceous plants there is a *secondary migration* from strawberry plants to other herbaceous plants.

The bugs feed on strawberry leaves for a few weeks and then leave them for other herbaceous hosts.

In 1926 at Bluntisham the number of bugs feeding on strawberries began to decrease towards the end of May, and by early June it was difficult to obtain bugs from these plants although they were abundant on neighbouring weed hosts.

A similar migration was noticed in early June in 1927 both at Wisbech and Bluntisham and by June 18th practically all the bugs had left the strawberries.

At Wisbech and West Norfolk the dates of the emergence of the various instars were practically the same as those at Bluntisham and Cambridge. In all the plantations visited at the two former places the damage on June 1st, 1926, was distinctly worse on red currants than on black currants, in most cases the attack on the latter being comparatively slight. At this stage herbaceous plants was their favourite food. It was difficult to make any estimates as to the numbers of bugs present from time to time, owing to the difficulty of obtaining them by beating and sweeping in cold and wet weather. On warm sunny days the bugs were very active and could easily be shaken from the bushes, whereas cold wet weather makes them sluggish and they apparently feed but little.

The adults which were present throughout June and July were very active in warm weather flying readily from plant to plant, in which respect they differ from *P. rugicollis*. When shaken into a beating tray they would quickly fly back to the bushes. Towards the end of June the bugs became scarcer and scarcer on the currant and gooseberry bushes till by July 1st at Bluntisham it was almost impossible to find a single bug on bushes where a few weeks before we had found hundreds. In 1927 a small proportion remained on the currants and gooseberries and laid eggs there.

In 1926 egg-laying took place from the third week in June till the end of July, and the first bug of the second brood was found on July 13th. Summer eggs were found on potato, groundsel, white dead-nettle, *Urtica urens*, *Convolvulus arvensis* and *Convolvulus sepium*.

In cages eggs were readily laid on strawberry, shepherd's purse, *Urtica dioica* and apple shoots. First stage instars were also found on artichokes. Rostrup and Thomsen record eggs on potatoes, beans, etc. The young of this brood feed almost entirely on herbaceous plants; in cases where a currant or gooseberry bush had formed succulent shoots at

this stage damage was found. One egg was found laid very obliquely in the old wood of a currant showing that if sufficient succulent leaf be available eggs may be laid and young of summer brood reared on currant. In 1927 first instars of this summer brood were found on currants, gooseberries and artichokes. An attempt to rear young bugs of this brood in a sleeve on older, but by no means hard, currant leaves failed completely, no sign of spotting being observed. (This suggests that an additional reason for absence of this brood from currants is that the young bugs often find the available food supply unsuitable.)

The favourite food plants at Histon were *Ranunculus repens*, groundsel and *Urtica dioica*. The first adult of the second brood was observed on August 9th and no immature bugs were seen after August 30th. In September the presence of the adults on currants appeared to be mainly for the purpose of egg-laying, most of the feeding being done on herbaceous plants. Bugs were obtainable by sweeping as late as October 8th in 1926 and October 20th in 1927.

We have no exact figures for the dates of the autumnal egg-laying, but it probably commenced about the end of August and continued till early October, egg-laying was seen on September 14th, 1926. Bugs dissected in early October still contained eggs apparently ready for laying. The eggs laid at this time live through the winter and hatch the following spring.

In addition to currants and gooseberries, eggs are also laid in the autumn in apple shoots. They are also probably laid in plum suckers, and the shoots of pears, cherries, rose[1], raspberry, *Ribes aureum*, lilac, hawthorn and snowberry (*Symphoricarpus*), as first stage instars are found on these early in the season. We also found second stage instars on peaches. We also regard the blackberry as a probable winter host, as this plant is attacked by both generations and is largely used as a food plant by the second generation adults. Careful search failed to reveal any autumn eggs on herbaceous plants, the return to woody plants for this purpose being complete.

In 1927 an interesting case of migration occurred near Wisbech. In a mixed plantation the gooseberries (no currants present) were very badly attacked early in the season. Early in May, whilst in the second instar stage, migration took place to the strawberries between the rows of gooseberries. Later on they were found on herbaceous weed hosts. They continued to feed on the gooseberries, strawberries and weeds until the beginning of July, but in the second week in June the adult bugs

[1] We have now found eggs in both climbing and bush roses.

began to migrate to potatoes in the neighbouring field on the east side separated by a dyke. By the first week in July migration to the potato field was almost complete. On this date only a few bugs could be found in the mixed plantation, whereas hundreds were present in the potato field. The rows of potatoes nearest the mixed plantation were very badly damaged by the bugs, further away the attack became gradually less but extended for about 30 yards.

The second generation of bugs were abundant on the potatoes, but when they reached the adult stage in August they migrated, some back to the gooseberries and large numbers to a nursery and a young mixed plantation across a railway line on the south side. Here they attacked apple and apple stock, plums, cherries and cherry stock, and roses, and a number of eggs were laid on young apples.

Proportion of Sexes.

In both broods the females were found to be very much in excess of the males. In the summer brood the figures were approximately 30 per cent. and 70 per cent., while a count of 60 insects of the autumn brood gave closely similar figures, namely 28 per cent. and 72 per cent.

Time and Nature of Injury.

The chief cause of the excessive damage done by *L. pabulinus* to the various host plants is due to the fact that the saliva which is injected into the plant tissue at the time of sucking is very toxic as is the case with that of *P. rugicollis*. This has been confirmed experimentally by the work of Mr K. M. Smith (17) and others.

Similar injury is caused by all the active stages of the bug in both generations.

The greatest injury to fruit trees and bushes is caused firstly by the young stages of the first generation, feeding continuing voraciously on these plants from the time of hatching in April till the time of migration, and secondly by the adult stage of the second generation which returns to these plants. Under certain conditions a small proportion of all stages of both generations remain on the fruit hosts and cause damage.

The bugs always choose the youngest and most succculent part of the plant available and consequently cause much greater injury to the host plant than would be the case if they fed on the older parts.

Damage to currants. Red currants usually suffer more severely than black currants but the damage is very similar. On young leaves the sucking of the early instars produces at first minute, almost transparent, dusky brown spots which, as the leaf grows, form holes with brownish edges.

In the case of a very bad attack the young leaves may be killed without ever becoming fully expanded, especially in the case of red currants. In the early stages, at least, the greater part of the feeding appears to be done from the upper surfaces of the leaves where the larvae are more sheltered within the developing shoots, but in the later stages the bugs appear to feed equally freely from either surface. Spotting is most frequent on the basal half of the leaf and in the region of the mid-rib rather than at the leaf edges.

On slightly older leaves the early spots are not so conspicuous but the light spots are more noticeable, especially when viewed by transmitted light. As mentioned above when a young leaf is badly attacked its further growth may be entirely prevented. If, however, the intensity of the attack is insufficient to kill the leaf, growth continues and the dead areas killed by the bug fail to expand with the result that the leaves are riddled with irregular holes. A bad attack often causes side-shooting, *i.e.* a number of buds in the current year's growth develop into side shoots (see Fig. 12). This effect may also be produced by other factors which cause injury to the top of the developing shoot. This side-shooting has been more noticeable in red currants.

As far as our observations go but little *direct* damage seems to be done to the fruits of currants. That the bugs do occasionally suck the berries causing the formation of a brown scab seems probable, but larvae in cages showed no signs of feeding on the fruits. Greater readiness was shown to feed on the fruit of the gooseberry.

The bulk of the injury is done by the early stages of the first generation.

The feeding of these bugs on the shoots appears to be responsible for a marked reduction in the crop, and numerous complaints have been received from growers in the Eastern Counties. Mr H. Goude, the Horticultural Superintendent for Norfolk, considers this pest a big factor in some of the recent low yields of black currants in Norfolk, taking into consideration the losses due to frost and aphides.

We have seen red currants growing on good land, which produced a very poor crop indeed after a bad attack of this pest, and in addition the bushes made very little growth except side-shooting.

Damage to gooseberries. The early stage damage appears as dusky brown patches on the young leaves which often crack as the leaf gets older. On the older leaves the damage may appear as raised pale green spots on the upper surface with a corresponding indentation on the lower surface. As the leaf grows these develop into characteristic holes similar to those on currants. In the case of a bad attack a fairly large percentage of the leaves shrivel and fall off.

Injury to the fruit is of a very serious nature. The damage first shows itself as brownish yellow patches underneath the skin; later the berry becomes dimpled and a dark brown cracked and scabbed area may form. Injured fruit often falls off.

In addition to the direct injury the damage to the growing tip causes a number of the buds in the present year's wood to grow out into shoots of varying length (see Fig. 13). Many of the leaves on these shoots are abnormal in shape having very much fewer serrations than those of a normal leaf. This change is reminiscent of that of the black currant leaf on a reverted shoot.

The injury to the tip of the gooseberry shoot by the bug causes similar side-shooting as does early "tipping," consequently it is very difficult from a winter examination of gooseberries to decide if the side-shooting is the result of a capsid attack or not, as any damage to the tip early in the season would produce the same result.

Damage to apples. The young bugs which hatch from the eggs on the apple shoots soon begin to feed on the young developing leaves causing brown spots similar to those caused by *P. rugicollis*. This damage is usually rather later than that of the apple capsid. In 1926 and 1927 these bugs were found one day and twelve days respectively later than the first found bugs of *P. rugicollis*.

These bugs continue to feed on the leaves but usually migrate in the later instars. They also cause a certain amount of damage to the shoots at this stage and these injuries later on form scars similar to those of *P. rugicollis*. Further damage to the leaves and shoots is also caused by the adults of the second generation which return to the apples from herbaceous hosts usually in August and September. A few nymphs of the second generation were also found on apples. The adults suck the shoots causing a brownish fluid to exude which dries and leaves an orange-red stain over the wounds. These wounds later on form scars. This damage is similar to that caused by *P. rugicollis*, but is later in the season, being caused long after the apple capsid is dead.

We have no evidence as to the damage caused to the fruits. We sleeved a number of bugs on apple shoots but the apples failed to set. Moreover, the trees on which we found *L. pabulinus* and not *P. rugicollis* also failed to set fruit probably due to frost. Rostrup and Thomsen (13) state that *L. pabulinus* suck young apple fruits in Denmark.

L. pabulinus taken from currants and sleeved on healthy apple shoots caused markings similar to those of *P. rugicollis*, although the average size of the spots appeared to be rather smaller. Rostrup and Thomsen (13)

further state that "as an apple pest it plays hardly as large a part as *P. rugicollis* in Denmark."

Damage to plums. Characteristic brown spots and later holes are produced on the leaves and also some scars on the stems similar in appearance to those on apples. Suckers usually suffer much more than worked nursery trees and we rarely found any damage on older trees.

Damage to pears. The damage was found on grafts and also on trees. Small, almost black, spots are found on the leaves. These are often in lines due to the feeding of the bugs on the curled edges of the young unfolding leaves. As the leaf grows these spots form holes with yellowish brown edges. K. M. Smith(17) also says that they cause dark spots on the fruit. We have seen scars on the fruit probably caused by this pest and Fryer (*in litt.*) recorded this in 1914.

Damage to strawberries. Damage to young strawberry leaves very similar to that on young leaves of *Ribes* sp., the spots being dark brown. This is caused by the young bugs (usually starting in the second instar) migrating[1] from woody host plants at the end of April or early May. In June the bugs leave the strawberry for other hosts. A few bugs from the second generation also damage strawberry leaves.

The only case we have noted of *P. rugicollis* injuring strawberries was a single specimen on May 2nd, 1927, attacking a strawberry plant under an infected apple tree.

A few specimens of *Calocoris bipunctatus* in the first instar were found in the middle of a strawberry field near Wisbech causing similar damage to that of *L. pabulinus*.

Damage to raspberries. The damage on the young leaves consists of minute brown specks which is much less conspicuous than on the previous hosts mentioned.

Damage to blackberries. Blackberry leaves were badly damaged by the second generation, in many districts the brown spots forming the characteristic holes. The stems were also marked. The adults also suck the fruits. Both generations cause damage and in places the bug appears to live on this host without migrating.

Damage to roses. Both generations cause brown spots and later on characteristic holes on wild roses, all the rose stocks examined (a large number), and also bedding roses and climbers (see Fig. 9).

In 1927 the second generation adults caused serious damage to the buds as well as to the leaves. Some buds were killed and on opening

[1] It is also probable that a few eggs were laid in strawberries in the autumn and that a few bugs hatch from these in the spring.

them the petals had turned black. After a bug has sucked a bud through or between the sepals a drop of dark fluid exudes; underneath this a large area of the petals turns black. Sometimes the buds open but are deformed. Carpenter (5) recorded this damage in Ireland in 1911. On September 8th, 1927, at Wisbech, 8 adults were present on a shoot less than a foot long. Mr F. Glenny of Wisbech, a well-known rose grower, says his roses have suffered from this capsid since 1914.

Damage to potatoes. The later stages of the first generation and all the stages of the second generation cause damage by feeding at the growing point. Brown spots of comparatively large size are formed on the developing leaves, and later on these form the characteristic holes with yellowish edges.

If the leaf is attacked when very young the lower leaflets fail to develop properly and the leaf area of the plant is very much reduced with a consequent reduction of the crop.

Plants near badly attacked fruit bushes can suffer severely.

Damage to runner beans and dwarf beans. These sometimes suffer badly in allotments and gardens containing infested fruit bushes. The young developing leaf is marked with brown spots which as the leaf grows forms holes, which often coalesce, causing malformation and riddling of the leaflets.

The bugs also feed on the young developing pods before they are an inch long and also later, causing brown spots, which later form brown warts or corky areas or light coloured spots. Badly attacked pods often fail to develop and when they do they are malformed and curved. In some cases observed, the crop was greatly reduced.

Damage to broad beans. The young unfolded and unfolding leaves are marked with moderately large, irregular, almost black spots. These later form irregular holes with brown margins. The leaves are often badly injured.

Damage to bindweed. The spots in this case are semi-transparent and later characteristic holes are formed. These plants are readily attacked and an examination of them is often a useful guide as to the prevalence of this pest.

K. M. Smith (17) states that he knows of no case in which a capsid that was harmful to one plant was harmless to another, but says that the damage may vary slightly according to the reaction of the plant juices to the saliva.

The difference in the reaction of various plants to the feeding of *L. pabulinus* is very marked. Some plants like potatoes and bindweed and currants are severely marked while groundsel and stinging nettles,

although extensively attacked, show very little damage. In a mixed patch of stinging nettles and dead nettles the latter can often be easily detected by the amount of damage.

On several occasions *L. pabulinus* was found on *Chenopodium album* (fat hen) but we found no typical markings. A pale spotting found on this plant may be due to this bug or to *Orthotylus flavosparsus* which was common on this plant in many localities.

The markings on the other host plants consist of the typical spots and subsequent holes.

In herbaceous borders the leaves of dahlias and *Salvia splendens* were badly damaged by the second generation and suffered much more severely than other host plants, although fuchsias also suffered considerably at the same time.

Host Plants.

The following lists show the plants on which we have found damage by *L. pabulinus*.

Cultivated plants. Apple and apple stocks, *Achillea ptarmica*, *Anchusa italica*, artichoke (*Helianthus*), bean (broad), bean (dwarf), bean (runner), beet (sugar), blackberry (parsley-leaved) (*Rubus laciniatus*), *Chenopodium bonus-henricus*, cherry and cherry stocks, chrysanthemums, *Chrysanthemum maximum*, clarkia, clematis, cosmia, black currant, red currant, dahlia, *Diervillia lutea*, *Forsythia viridissima*, fuchsia, gallardia, *Geranium* sp., godetia, gooseberry, grape (outdoor), hawthorn (occasional attack), helichrysum, hops (only a few bugs found), king acre berry, lavatera, lettuce, *Leycesteria formosa*, lilac, mangold, marigold (French and African), *Melissa officinalis*, michaelmas daisy, mint, new berry, oenothera, peach, pear, pelargonium, phenomenal berry, physalis, poppy (Shirley), potato, potentilla, privet (two cases of slight damage), raspberry, *Ribes aureum*, rose and rose stock, *Salvia splendens*, scabious, snowberry (*Symphoricarpus*), strawberry, sunflower (*Helianthemum*), valerian, *Viburnum opulus*, weigelia, Worcester berry.

Weeds. *Aegopodium podagraria* (ground elder), *Carduus arvensis* (creeping thistle), *Convolvulus arvensis* (lesser bindweed), *C. sepium* (larger bindweed), *Humulus lupulus* (hop), *Lamium album* (white dead nettle), *L. purpureum* (red dead nettle), *Malva sylvestris* (common mallow), *Polygonum amphibium* (amphibious polygonum), *P. persicaria* (persicaria), *Ranunculus repens* (creeping buttercup), *Rosa canina* (dog rose), *Rubus fruticosus* (blackberry), *Rumex crispus* (curled dock), *Sambucus nigra* (elder), *Senecio vulgaris* (groundsel), *Solanum dulcamara* (bittersweet), *S. nigrum* (black nightshade), *Sonchus oleraceus* (common

sow-thistle), *Stachys ambigua*, *Symphytum officinale* (common comfrey), *Taraxacum dens-leonis* (dandelion), *Teucrium scorodonia* (wood sage), *Urtica dioica* (common stinging nettle), *U. urens* (small stinging nettle).

The extent of this list suggest that this pest feeds on almost any available plant, but our observations show that this is not the case. No sign of damage was ever observed on *Stellaria media* (chickweed), *Capsella bursapastoris* (shepherd's purse), or the Veronicas although they were abundant in the flora in which the bugs were numerous. Moreover, in the case of herbaceous hosts the bugs show a decided preference for some species, *e.g.* potato and bindweed, and in hedges show a preference for blackberry and rose over such hosts as hawthorn and privet.

It has previously been recorded on the following plants:

Reuter (11)—*Rubus idaeus*, *Chenopodium*, *Atriplex*, *Aspidium*. Dalla Torre (7)—alders and willows; Butler throws doubt on this but K. M. Smith (17) records damage on willows in addition to several of the above hosts. Sahlberg (15)—*Spirea ulmaria*. Schoyen (16)—oats. Ritzema-Bos (12)—guelder rose. Butler (3)—*Epilobium angustifolium*. Fryer—dahlias and pears. Rostrup and Thomsen (13)—apples, potato, blackberry, beans, chrysanthemums, and dahlias.

Carpenter (5, 6) records damage to beans, peas and roses, and also serious damage to apples in Armagh, but concerning this latter record (*in litt.* 1926) says "in view of subsequent work on the subject there can be no doubt that *Plesiocoris* was the real agent."

Control.

This pest is readily killed by contact washes in the same way as *P. rugicollis*, but there are difficulties in reaching the bugs with the wash.

They readily fall to the ground and the currant bushes form such a dense mass of foliage that the spraying of bushes sometimes gives rather disappointing results. The method which has given the best results is first to shake the bushes and then spray the ground very thoroughly underneath the bushes.

In order to have its maximum effect the wash should be applied after all the bugs have hatched. The actual date will vary from year to year, but is about the time that Bramley's seedling apple is in full bloom. If spraying is delayed much later than this, some of the bug may have migrated to strawberries and weeds. When spraying is delayed weeds should be kept down to prevent this migration. At present the best wash to use is nicotine, 0·05 per cent. of 95–98 per cent., *i.e.* 8 oz. to 100 gallons, or its equivalent, together with a spreader. With soft water

and ordinary waters which do not form much scum, soft soap at the rate of 8-10 lb. to 100 gallons of water is the best spreader to use. With very hard waters where soft soap is uneconomical, sodium caseinate or other suitable spreaders may be used.

A high-pressure pump and a coarse nozzle should be used for spraying.

Spraying during the second generation on currants and gooseberries is hardly likely to be profitable as the bugs are also present on neighbouring hosts.

When weed hosts are prevalent it may prove profitable to leave these until the eggs are laid in them, *i.e.* until the first instars of the second generation are found in the middle of July or rather later, according to the season, and then fork them in.

In nurseries when this pest is more likely to be troublesome a trap crop such as potatoes might be used. The eggs will be laid in the potato tops and weeds, and these could then be cut off and destroyed as soon as the young bugs of the next generation are found.

In the case of apples, trees should be sprayed immediately after blossoming and the ground round the trees also sprayed.

It is of no use to knock these bugs off the trees and grease-bands as has been suggested for *P. rugicollis*, as these bugs, unlike *P. rugicollis*, normally live on herbaceous hosts, usually found growing underneath.

In small private gardens a cheap method is to shake the bugs off on to trays made from an old sack (or other material) or into an open umbrella and destroy them, or to shake them on to sticky trays.

Following on the lines of Andrews' (1) work on the tea mosquito bug, an attempt was made to find what effect an increase in the proportion of potash had on the feeding of the bugs.

Potatoes growing in sand were watered periodically with a *complete* food solution to which 1 per cent. of potassium nitrate was added. Other potatoes were similarly treated except that 1 per cent. of sodium nitrate was substituted.

No difference whatever was noticed in the feeding of the bugs on the two sets of plants.

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Summary.

The capsid bug *Lygus pabulinus* has become a serious pest of currants, gooseberries, strawberries, potatoes and dahlias, and is also present on apples, pears, plums, cherries and peaches, and a large number of cultivated plants and weeds.

A detailed account is given of the characteristics and measurements of the various stages which will serve the purpose of identification, and also of the genitalia.

Diagnostic characters are given for the purposes of distinguishing it from the *P. rugicollis* which causes similar damage on currants and apples.

There are two generations per annum. Eggs are laid in the autumn in woody plants such as currants, gooseberries, apples and roses and then migration to herbaceous hosts usually takes place where the summer eggs are laid, the second generation returning to the woody host to lay their eggs.

In the first generation a secondary migration from strawberries to other herbaceous hosts takes place. Details are given of the damage done to the more important host plants and of the habits of the bug in its various stages. Control measures are given.

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Fig. 9. Rose leaf damaged by *Lygus pabulinus*.



A B

Fig. 10. A. Egg of *Lygus pabulinus*. B. Egg of *Plesiocoris rugicollis* (both dissected from stems).



Fig. 11. Damage to red currant leaves by *Lygus pabulinus*.

STUDIES ON *OSCINELLA FRIT* LINN.

OBSERVATIONS ON INFESTATION AND YIELD, SUSCEPTIBILITY TO INFESTATION, RECOVERY POWER, THE INFLUENCE OF VARIETY ON THE RATE OF GROWTH OF THE PRIMARY SHOOT OF THE OAT AND THE REACTION TO MANURIAL TREATMENT

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I. Introductory Remarks.

The broad aim of research work on the frit fly has been the establishment of varieties of oat plants resistant to the attack of the fly in spring and during the course of investigation within the last four years many possible explanations of observed differences in regard to resistance between varieties of oat plants have presented themselves. Some of these lines have been followed, so far as time has permitted, and the accumulated data either have been presented in previous publications or are incorporated in the notes included in the present publication. In view of their nature the latter have been presented in a very concise form, a short statement of experimental data and any conclusions derived therefrom being recorded without unnecessary amplification. The ground covered is sufficiently indicated by the table of contents.

II. A note on Infestation and Yield.

The study of the problem of *varietal* resistance to attack was commenced during the year 1923, and differences in the extent of infestation of shoots of the order of 20 per cent. have been recorded between *Goldfinder* and *Supreme* oats, in favour of the former variety, at Harpenden and Oxford, similar results being obtained again during the following year. Such data have been collected at the beginning of June, after a period reckoned to be sufficiently long to allow larval development to take place after the period of maximum infestation and before confusion between the damaged shoots and those which died naturally was likely to occur. By this time of year panicle production had hardly commenced, and separation of the shoots into producers and non-producers was impossible, but it was then considered that all shoots present in the field at the beginning of June had sufficient time allowance to attain maturity and, therefore, that all the shoots then present should enter into any analysis of extent of infestation. An obvious question was whether percentage differences of shoot infestation of this order and differences in yield were of the same order and sign; so, during the year 1925, the varieties *Goldfinder* and *Supreme* were used to determine whether there was any correlation between these values or whether the quality of a variety would compensate for heavy infestation.

The best selected commercial seed was sown on April 1st and 2nd inside a wire cage, 50×30 ft., for protection of the grain against birds and mice, plots of five rod-rows of each variety being put down in the varietal order *A*, *B*, *B*, *A*, *A*, *B*, etc., and the seed spaced at $2 \times 6 \times 1\frac{1}{2}$ in. by means of a sowing-board. In this way ten pairs of rod plots in two blocks were prepared and enough extra seeding was carried out at the extreme ends of the plots to allow for sampling for the estimation of frit infestation. Cover rows of *Abundance* oats were sown all round to a width of 2 ft. The plants commenced to show on April 15th and germination was even. During the growing season, four pairs of the plots were seriously damaged by the working of moles before the latter could be trapped and consequently were discarded at harvest time.

The examination of the shoots for frit infestation was conducted on June 8th and 9th, the plants included within a foot of each of the three centre rows being pulled for this purpose, the numbers of plants, the numbers of shoots and the numbers of the infested shoots being recorded. The minimum, mean and maximum numbers of individuals examined from a rod row sample were as follows: *Goldfinder*, plants, 8, 14 and 18;

shoots, 43, 65 and 93: *Supreme*, plants, 6, 12 and 18; shoots, 30, 46 and 70 respectively, there being twenty samples of each variety. The three centre rod-rows were harvested from each plot on August 5th and 6th, and the numbers of plants and panicles recorded. During the winter the grain was threshed out by means of a simple contrivance constructed of cartridge paper and an ordinary electric fan, then weighed and sampled for frit infestation. The minimum, mean and maximum numbers of grains examined per plot were, for *Goldfinder*, 670, 797 and 895; and for *Supreme*, 781, 827 and 862 respectively. The significance of the differences has been determined by Student's method, and the degree of significance is shown in the last column of the following synopsis of the data obtained.

All the differences, excepting that for the mean yield of grain per plot, are well above the usual standard of a 30 to 1 chance.

Synopsis of data.

	Gold-finder	Supreme	Data relating to the significance of differences		
			σ	x	Odds
Mean number of plants per plot at harvest	1·97	1·57	8·8	·9999	9999
Mean number of shoots per plant on June 8th–9th	4·75	3·72	0·35	·9993	1428
Mean percentage infestation of shoots	30·3	49·5	10·7	·9945	181
Mean numbers of panicles per plant	1·57	1·42	0·11	·9852	66
Mean percentage infestation of seed	14·0	10·4	2·54	·9876	80
Mean yield of grain per plant in gm.	3·00	3·43	0·32	·9851	66
Mean yield of grain per plot in gm.	591	538	60	·9442	17

The difference in the yield of grain was not significant, although all the contributory factors, excepting extent of seed infestation and yield of grain per panicle, were favourable to *Goldfinder*. The absence of any difference in yield must therefore have been due to biological factors other than the infestation, their influence culminating in the difference in panicle production per unit area and the difference in weight of grain produced per panicle. The slight difference in extent of seed infestation cannot have had much influence on the weight of seed produced in this particular case. There was, therefore, no correlation between the difference in percentage infestation of shoots and the difference in yield, even though the former was of the order of 20 per cent. From the 285 seeds sown in the three centre rows of each plot, *Goldfinder* and *Supreme* produced plants as follows: by June 8th, 77 per cent. and 67 per cent. of the possible numbers; at harvest time, 69 per cent. and 55 per cent. of the possible numbers respectively. Between these dates, *Supreme* lost

slightly more plants than *Goldfinder*, the loss no doubt being mainly due to the fly. The mean numbers of shoots produced per plant in June were 4·57 for *Goldfinder* and 3·72 for *Supreme*. Thus within equal areas, under what appeared to be identical conditions, the two varieties produced very different mean numbers of shoots by June 8th (*Goldfinder* 934 and *Supreme* 582), but presumably each as many as it could under the particular conditions of the experiment. The significant difference, demonstrated by the proper arrangement of the plots and subsequent statistical analysis, was due to differences in reaction to the particular environment. The actual mean numbers of infested shoots were identical in the two cases (*Goldfinder* 282 and *Supreme* 288), showing either that the number of the larvae was limited over the two areas or, if the number was unlimited, that resistance factors had operated to produce this result by a coincidence. There is, however, no direct evidence to differentiate between either contention in this particular experiment. In this case, *Supreme* presented a lesser number of shoots for attack by the fly population than did *Goldfinder*, and therefore it lost a larger percentage of shoots, namely 49·5 per cent. against 30·1 per cent.

Although the mean numbers of shoots per plant in June were 4·75 for *Goldfinder* and 3·72 for *Supreme*, the panicle production was very limited, even under such wide spatial conditions, being only 1·57 and 1·42 per plant respectively, and therefore not in any way equivalent to the shoot production in June, even after making due allowance for the frit infestation. There was a great wastage in shoot production, because, of the total numbers of shoots produced by June 8th, 36·7 per cent. of those of *Goldfinder* and 12·2 per cent. of those of *Supreme* failed to produce panicles, even though they were not infested at that date. Many of these shoots died a natural death and others doubtless were killed by later frit attacks. Probably some replacement occurred by later tillering also. Detailed records of such complicated changes in the shoot population are lacking because of the difficulties involved in marking and observing the histories of innumerable individual shoots. The shoot population was greatly lacking in uniformity but, of necessity, productive shoots counted equally with unproductive shoots in the measurement of the difference in percentage infestation.

As the actual numbers of infested shoots were the same in the two cases, it may be argued that a difference in resistance was not measured, and therefore that the percentage difference in infestation as thus measured has no real meaning, being merely a statistical difference. It is true that differences in direct resistance have not been measured, but it

is equally true that differences in direct resistance plus indirect resistance have been measured and that such observations have a definite meaning under particular environmental conditions, which determine the size of the fly population and the condition of plant growth. The quality of a variety may therefore compensate for comparatively heavy infestation.

III. ADDITIONAL NOTES ON THE SUSCEPTIBILITY OF THE OAT SHOOT TO INFESTATION.

During the years 1923 and 1924 observations were made on the correlation between the stage of growth of the oat shoot and its susceptibility to infestation by the frit fly (1). As no other data on this subject have been published it seems desirable to record, in the form of a short note, the results of a repetition of those experiments conducted during the year 1925. Exactly the same general experimental procedure was adopted and does not require further note. In the spring of 1925, each of three cages was arranged to contain six drills (numbered 1–6 below) which were sown with *Abundance* oats on March 26th, April 9th, April 23rd, May 7th, May 21st and May 28th respectively. The cages were removed on May 14th, May 21st and May 28th, and replaced on May 21st, May 28th and June 4th respectively and the leaf stages of the shoots were recorded both on removal and on replacement of the cages. The minimum, mean and maximum numbers of plants per drill were 60, 81 and 116 respectively. The following data were obtained from the plants in each cage, when they were examined three weeks after replacement. As before, the letters E, M and L denote early, middle and late types of a leaf stage.

It is very difficult to forecast the effect of weather conditions on growth and for this reason, together with the fact that the cage space available was very limited, we again failed to get plants beyond the five leaf stage at the time of exposure. However, the observations are entirely confirmatory to those previously published, the very rapid decrease in susceptibility after the four leaf stage again being most marked. That the differences are for the most part significant may be determined easily from the standard errors.

During the year 1926 our knowledge of the correlation between leaf stage and susceptibility was utilised to determine whether an oat crop was likely to be seriously damaged by the population of flies present in the field before the period of maximum prevalence, *i.e.* before the end of May. For this purpose it was obviously most desirable to utilise plants the primary shoots of which were in the three leaf stage. As it

Cage 1.

Drill	Leaf stage on removal May 14th	Leaf stage on replacement May 21st	Percentage infestation and standard error
1	3+4 E	4+5 E	13·4±4·2
2	2+3 E	3+4 E	28·8±5·3
3	1	2+3 E	59·6±5·5
4	None showing	2	47·8±4·7
5	—	Sown as control	0·0

Cage 2.

Drill	Leaf stage on removal May 21st	Leaf stage on replacement May 28th	Percentage infestation and standard error
1	4+5 E	5	10·2±3·6
2	3+4 E	4	27·4±5·9
3	2	3	32·0±5·5
4	1+2 E	2+3 E	71·7±5·5
5	Sown May 21st	Many not showing	6·9
6	—	Sown as control	0·0

Cage 3.

Drill	Leaf stage on removal May 28th	Leaf stage on replacement June 4th	Percentage infestation and standard error
1	5	6+7 E	14·0±3·7
2	4	5	28·2±4·9
3	3	4	68·1±6·0
4	2	3+4 E	69·3±5·9
5	Not showing	2 E	97·3±1·9
6	Sown May 28th	1 E	28·5±4·2

was impossible to forecast the leaf stage which would be reached during any particular period, it was necessary to sow a number of plots, at intervals of about three days, and to commence sowing about the beginning of March. Sowings were made at the normal rate of $1\frac{1}{4}$ gm. per foot of drill, the drills being spaced at 9 in., on plots 6 ft. square, and the first shoots were marked by a spray of Indian ink, suitably diluted.

Plots carrying plants which reached the medium three leaf stage on May 1st, 8th, 15th and 24th were screened for fourteen days and the plants then examined for infestation without further exposure.

Plants covered during the periods May 1st to 15th, May 8th to 24th, May 15th to 29th and May 24th to June 7th gave the following data: sown March 22nd, March 29th, April 19th and April 26th; numbers of plants 661, 913, 775 and 769; leaf stages 3 M, 3 M, 2 L and 3 L; percentage infestation of first shoots, with standard errors, $0{\cdot}5 \pm 0{\cdot}9$,

1·9 ± 0·4, 5·8 ± 0·8 and 55·2 ± 1·8 respectively. Thus, before May 15th, the infestation was negligible, while during the period May 15th to 24th the severity of attack increased greatly, namely from 5·8 per cent. to 55·2 per cent. During the early part of the emergence period the flies were not sufficiently numerous to cause damage of economic importance, but, just before the maximum prevalence period, plants in a susceptible stage suffered severely.

IV. A NOTE ON THE EXTENT OF THE RECOVERY POWER OF "SUPREME" OATS WHEN SUBJECT TO INJURY.

During 1924 some observations were made on the oat varieties *Sir Douglas Haig* and *Scotch Potato* in relation to the problem of recovery after injury(2). During the following year *Supreme* oats, which in England is the variety most susceptible to damage by the frit fly, was subjected to similar treatment on a somewhat more extensive scale.

Selected seed was sown in the centre of an insectary in the garden of the School of Rural Economy on April 19th, 1925, spacing and depth being controlled by the use of the sowing-board, the seeds being $1\frac{1}{2}$ in. deep and 2 in. apart in the rows and the rows themselves 6 in. apart. Cover rows were sown all round to a depth of $1\frac{1}{2}$ ft. On the thirteen rows sown (each with eighteen seeds), seven alternate rows were left for controls, and the remaining six were treated in exactly the same way as were *Potato* and *Haig* during the previous year, except that the first shoots alone were killed, and subsequent tillers were left undamaged. The growing points were killed in the middle of May when the shoots were in the three leaf stage, that is, in a condition when they would be most susceptible to attack in the field. The plants were pulled by hand on August 26th and dried in a greenhouse, the grain being threshed out by hand. As is always the case with cereals grown in an insectary the plants during growth were not vigorous and were very subject to attack by mildew, *Erisyphe graminis*, necessitating frequent spraying, and they were weakly when mature.

The numbers of plants in the control rows were, in order, 15, 14, 14, 15, 13, 6 and 16; the mean yields of grain per plant being 0·44, 0·84, 1·63, 1·93, 2·55, 2·73 and 1·75 respectively. The data for the treated rows, in order, were as follows: numbers of plants, 11, 13, 12, 13, 12 and 14; and the mean yields of grain per plant were 0·20, 0·49, 0·58, 0·35, 0·65 and 0·64 respectively. The mean yield per plant of each treated row was compared with the corresponding mean figure for the two control rows immediately adjacent and the differences were examined for significance

by Student's method. The mean difference in yield in grams per plant was 1·31, δ being 0·576, therefore x was 0·9985 and the chances of significance about 600 to 1. The reduction in yield, owing to the treatment, was therefore of the order of 70 per cent.

It is difficult to say how far insectary conditions influence recovery power, but the plants are certainly not placed in the most favourable conditions to enable them to withstand a severe check, so that probably such experimental data bears but little relation to field data as far as actual values are concerned. On the other hand, these experiments show that different varieties may react very differently and they indicate the importance of arranging field conditions to favour the growth of the primary shoots rather than that of all the shoots and consequently the importance of using only primary shoots for determining the extent of damage by frit fly.

This conclusion is confirmed by the following observations, which show that uninfected spring oats of the grain-producing type, do not tend, under normal commercial conditions, to produce tillers of commercial value. During the years 1925 and 1926 samples were taken from commercial crops of *Victory* oats, from what appeared to be the most normal areas of the fields. The plants were lifted carefully, conveyed in sacks to the laboratory, and there examined individually. The early collection of the first three samples made it possible to separate the

Synopsis of data relating to panicle production in the field.

(Figures in italics indicate number of spikelets per panicle.)

Sample no. (The names of the samples are given below)	No. of plants in sample	% no. of plants with 1–4 shoots. (One panicle only)				% no. of plants with 2–4 panicles (Shoots per plant variable)			% no. of attacked plants	
		1 shoot	2 shoots	3 shoots	4 shoots	2 panicles	3 panicles	4 panicles	With panicles	Without panicles
1	114	85·6	—	—	—	4·0	1·6	0·8	—	8·0
2	74	50·0 *19·8*	24·3 *36·2*	—	—	5·4 *67·2*	—	—	14·9 *17·4*	5·4
3	37	37·8 *13·7*	29·7 *31·0*	—	—	5·4 *69·0*	2·7 *100·0*	—	13·5 *10·9*	10·6
4	236	60·0 *12·4*	21·2 *20·0*	12·3 *24·4*	2·1 *20·0*	1·7 *24·7*	1·3 *39·6*	1·4	Included with others	
5	248	82·5 *11·7*	13·7 *23·7*	—	3·8	None with more than one panicle				

1. *Victory*, Sandford, July 1st, 1925.
2. *Victory*, Coombe, July 10th, 1925.
3. *Newmarket*, Coombe, July 10th, 1925.
4. *Victory*, Sandford, August 9th, 1926.
5. *Victory*, Sandford, August 9th, 1926 (2nd sample).

attacked plants with certainty, whereas with the 1926 samples it was not possible to do this, owing to the later sampling date, which accounts for the slight difference in tabulation in the synopsis given above of the observations made.

In the synopsis certain data are given as to the percentage numbers of plants bearing one to four panicles and the productivity of the panicles as measured by the numbers of spikelets produced. In each case quite 70 per cent. of the plants failed to produce more than one panicle and even of these by far the greater proportion only produced one shoot. From these figures, it will be seen that normally half the plants had not sufficient vitality to tiller at all. About one-quarter evidently had greater vitality because they produced one tiller and a much greater mean number of spikelets but, even so, they failed to bring the second tiller to maturity. Very few of the plants produced two or more panicles, but when they did, the plants carried comparatively large numbers of spikelets. The limiting factor is, no doubt, spatial condition and it is of paramount importance to note that under normal commercial sowing conditions, this factor operates in such a way as to prevent successful tillering.

Thus, as the plant normally only produces one panicle, the inference must be that it is the primary shoot which is of vital importance commercially and therefore the one which must be used in all estimates of extent of damage by external agents, at any rate until we have gained further knowledge of a plant's power of recovery.

V. The influence of variety on the rate of growth of the primary shoot of the oat plant, together with data relating to tillering and panicle exsertion in relation to infestation by frit fly.

Having proved that variation in susceptibility to attack by the frit fly varied directly with growth within a variety, the next step was to determine whether the differences in resistance to attack exhibited by varieties of oats could be attributed to variations in rates of growth during the vital period, *i.e.* between the times of emergence from the soil and the appearance of the sixth leaves. This problem was studied in England during the years 1925 and 1926.

Investigation during the year 1925.

In this experiment sixteen varieties were used, the seed being sown in the garden of the School of Rural Economy at the board spacing of

2 in. between the seeds and 6 in. between the rows. It was assumed that such comparatively wide spacing would not produce abnormalities in growth before the appearance of the sixth or seventh leaves, this wide spacing being necessary to ensure accuracy in observation and record. The labour involved in observation made it necessary to limit the number of plants, so the seed was sown in two batches, on March 27th and April 18th, at the rate of twelve seeds per row, with seven replications in the case of the earlier sowing and a replication with the later sowing, the latter being intended merely to indicate whether time of sowing itself would induce variation in growth rates.

Records were kept of the growth of the individual plants, so that individual histories could be followed throughout. Observations were made every other day, weather permitting, and data of interest in relation to the frit fly problem were recorded as follows:

(1) Date of first appearance of new leaves, when they became visible externally in centre of primary shoot.

(2) Date of appearance of tillers externally.

(3) Date of appearance of panicles from sheath.

The varieties of oats used were *Supreme*, *Goldfinder*, *New Abundance*, *Superb*, *Marvellous*, *Yielder*, *Captain*, *Tartar King*, *Record*, *Leader*, *Waverley*, *Victory*, *Scotch Potato*, *Sir Douglas Haig*, *Black Tartar* and *Black Bountiful*, all the seed being selected by visual examination only from commercial samples. The minimum, mean and maximum numbers of plants observed were 67, 79 and 89 for the first sowing and 17, 21 and 23 for the second sowing respectively.

The data obtained from the experiment may be recorded very briefly.

First sowing, March 27th. The mean number of days, as determined from all the plants on all the plots, required for the plants to attain the successive leaf stages, one to seven, reckoned from the date of sowing, were 18·1, 26·9, 38·3, 44·6, 49·1, 55·7 and 63·1 respectively; the maximum variations from these means, in days, shown by the different varieties were 0·6, 1·9, 0·8, 1·5, 1·3, 1·5 and 1·3 respectively.

Second sowing, April 18th. The corresponding data, in this case for the first six leaf stages only were as follows: 16·4, 22·8, 28·2, 32·5, 39·1 and 43·4 days respectively; the maximum variations from these means, in days, were 1·0, 1·0, 1·6, 1·4, 1·9 and 1·4 respectively.

Investigation during the year 1926.

The previous experiment was repeated during the year 1926 with the varieties *Goldfinder*, *Supreme* and *New Abundance* to determine the effect of another set of climatic factors, very early sowing, and in the case of *New Abundance* only, more normal spatial conditions. The three varieties were sown in the same manner as that adopted during the previous year, except that *New Abundance* was also sown so that the seed touched endways in the drill (24 per foot), to determine whether spacing had any influence on early growth-rate. The minimum, mean and maximum numbers of plants observed under the wider spatial conditions were 90, 95 and 100 for the first sowing and 104, 112 and 118 for the second sowing, in each case the number of replications of plots being ten. With the more thickly sown *New Abundance*, the total numbers of plants observed were 657 and 672 for the first and second sowings, each series again being distributed over eleven plots.

First sowing, February 8th. The mean numbers of days, as determined from the data obtained from the more widely spaced varieties, required for the plants to attain the successive leaf stages one to six, reckoned from the date of sowing were 24·5, 34·3, 51·8, 57·4, 66·1 and 77·5 respectively; the maximum variations from these means, in days, shown by the different varieties were 0·3, 0·6, 0·5, 0·2, 0·9 and 1·0 respectively. *New Abundance*, with the thicker sowing, gave the following means for all plots, namely, 24·1, 33·6, 51·1, 57·5 and 66·9 days respectively, the sixth stage being omitted.

Second sowing, March 1st. The corresponding data from these plots were (*a*) varieties widely spaced, general means for leaf stages one to six, 23·1, 33·0, 39·4, 46·7, 57·6 and 65·7; (*b*) the maximum variations from these means, in days, 1·8, 0·1, 0·7, 0·4, 0·9 and 0·7 respectively. *New Abundance*, with the thicker sowing, gave the following means for all plots, namely 19·7, 33·0, 39·8, 48·7 and 60·6 days respectively, the sixth stage again being omitted. The shorter average period required by this series for appearance above ground was doubtless due to inaccuracy in gauging the depth of the drills.

These results indicate most clearly that there was no difference of any practical importance between the rates of growth of the primary shoots of these particular varieties, even under varied conditions of climate and density of sowing. Therefore differences in the susceptibility of the primary shoots to attack by the frit fly cannot be ascribed to differences in rates of production of the leaf stages. An obvious criticism

is that all these English varieties are too similar in type, being so closely interbred, for any marked differences to be expected, so further observations were made during a visit to Sweden in 1927, when an opportunity of observing varieties from widely different strains and localities presented itself. It is hoped to record these data in a future publication, but it may be stated here that they confirm the above conclusion.

Tillering and panicle emergence, 1925 *and* 1926.

It was a matter of interest to extend the observations on the primary shoots to gain some information as to the relative times of tillering and panicle shooting under these particular conditions, because they were conditions which have been used when determining differences between varieties in power of resistance to frit fly attack. Some of the records are presented below, as differences from mean values derived from all the observations on all the varieties, very briefly, because they indicate why certain correlations are or are not exhibited by data relating to resistance to attack by frit fly.

First and Second Series, 1925.

Time reckoned in days after date of sowing and differences from indicated mean recorded for each variety. Data for second (later sown) series in italics.

	1st tiller shown Mean 46·8 *Mean 38·3*		1st panicle emerged Mean 94·5 *Mean 78·4*		2nd panicle emerged Mean 95
1. Supreme	+4·0	*+1·2*	−0·3	*−0·3*	−3·0
7. Captain	+3·9	*+2·3*	−1·4	*−1·0*	−3·4
3. New Abundance	+2·5	*−0·2*	−1·8	*−2·8*	−2·0
8. Tartar King	+2·5	*+0·9*	−2·6	*−1·6*	−0·6
9. Record	+1·4	*−0·6*	+2·4	*+1·3*	+3·2
12. Victory	+0·6	*+1·1*	−0·2	*+0·7*	−0·1
2. Goldfinder	+0·3	*+1·0*	+1·6	*+2·9*	+3·0
4. Superb	0	*+0·7*	−0·9	*−1·0*	−2·9
11. Waverley	−0·6	*−1·0*	+1·3	*+2·3*	+1·3
15. Black Tartarian	−0·6	*−0·2*	+6·8	*+3·6*	+8·0
6. Yielder	−1·1	*−1·0*	−2·0	*−0·4*	−0·5
5. Marvellous	−1·3	*−0·8*	−4·7	*−3·0*	−3·0
14. Sir Douglas Haig	−1·3	*+2·8*	−3·8	*−0·8*	−2·8
10. Leader	−2·2	*−0·4*	−2·2	*−1·3*	−2·7
13. Potato	−2·4	*−2·4*	+4·6	*+3·6*	+8·0
16. Bountiful	−3·2	*−2·9*	−1·3	*−0·4*	−2·5

First series, minimum, mean and maximum numbers of plants, 64, 79 and 89.
Second series, minimum, mean and maximum numbers of plants, 16, 21 and 23.

First and Second Series, 1926.

	1st tiller shown Mean 62·4 *Mean 52·0*		1st panicle emerged Mean 136·7 *Mean 120·9*		2nd panicle emerged Mean 144·8 *Mean 122·9*	
1. Goldfinder	+0·8	*−0·3*	−1·3	*−2·4*	−1·0	*+0·2*
3. New Abundance	−0·1	*+1·2*	−1·8	*+2·9*	−2·7	*+0·4*
2. Supreme	−0·6	*−0·9*	+3·2	*−0·4*	+3·8	*−0·6*

First series, numbers of plants, 90 to 100.
Second series, numbers of plants, 104 to 113.

In general, the time of tillering never departed markedly from the mean, the greatest departure being only 4·0 days, and its occurrence was of course dependent very much on environmental conditions, as shown by the means recorded in 1925 and 1926 series. Additional tillers would be produced with as little variation as the first because of the length of the susceptible stage. A correlation between percentage infestations of primary shoots and of total shoots on a plot may therefore be expected and is in fact generally obtained. Time of panicle emergence was not correlated with time of shoot production, and the limits were rather wider, namely, between eleven and twelve days. Taking these facts in conjunction with the shortness of the periods of susceptibility it is obvious why shoot and grain infestations are not correlated.

VI. The effect of excess of nitrogen and phosphorus on resistance to frit attack.

It was shown in 1919–20 that nitrogenous manures were not likely to repay the cost of application on average land in England (3), but as excess of nitrogen is supposed to produce a decrease in power of resistance to insect attack it was desired to determine whether any decrease would follow an excess of nitrogen likely to occur in the field.

A piece of land on the University Farm, measuring 54 × 45 ft., on which oats would normally have been grown in the rotation, was manured evenly with sulphate of potash at the rate of 1 cwt. per acre on March 10th, 1926, and marked out into nine plots. Superphosphate was applied to the plots in sets of three at the rates of 6, 0 and 3 cwt. per acre. The whole piece was sown with *Supreme* oats on March 22nd, at the rate of 1 gm. to 1 ft. by means of a Planet drill, each plot carrying twenty-one drills. Sulphate of ammonia was then applied on March 24th to the plots in sets of three in the opposite direction to that used for the superphosphate, so that a non-manured plot was left centrally as a control plot.

The primary shoots of drills 4, 11 and 18 were marked by means of an Indian ink spray on May 6th and from June 10th onwards these shoots were analysed for frit attack, an average of 476 plants per plot being examined. The extent of attack varied per sample from 5·0 per cent. to 14·2 per cent., but there was no variation of any moment from plot to plot. The average extent of attack on the primary shoots was 7·4 per cent. and the greatest variation from this figure was only of the order of 0·4 per cent. Therefore, although the attack was very light, it would appear that an excess both of nitrogen and phosphorus had no influence on resistance to attack in this particular case.

In the case of the grain, however, nitrogen in excess did reduce the extent of infestation. Each plot was sampled in six places, and an approximate average of 600 grains were examined from each sample, with the following results:

Arithmetic mean percentage infestation with standard error.

Sulphate of ammonia per acre	Superphosphate per acre		
	6 cwt.	nil	3 cwt.
2 cwt.	3·8 ± 0·7	4·3 ± 0·4	3·8 ± 0·7
Nil	6·1 ± 0·8	9·3 ± 0·9	8·7 ± 0·7
1 cwt.	6·7 ± 0·6	7·2 ± 0·8	10·7 ± 0·8

If any one treatment had any real influence it should show throughout the series of three and should therefore be reflected by differences in the weighted means derived from the data supplied by the three plots similarly treated with one manure.

Taking the superphosphate series downwards, the weighted means are 5·63 ± 0·39, 5·67 ± 0·37 and 7·50 ± 0·41 respectively. The significant difference between the third mean and the other two is due to the last reading (10·7) but does not receive any support from them, and therefore cannot be explained except by soil heterogeneity.

Considering the nitrogen series across, the weighted means are 4·07 ± 0·32, 7·97 ± 0·44 and 7·95 ± 0·40 respectively. The reduction in the extent of attack to one-half in the first of the series and that compared with the equal means obtained from the other two series shows the marked effect of excess of nitrogen. The plots should of course have been replicated, but the necessary labour for analysis was not available.

VII. SUMMARY.

The notes collected in the present publication are each concerned with some aspect of the problem of production of varieties of oat plants resistant to the attack of the frit fly.

The quality of a variety may compensate for comparatively heavy infestation.

Data are recorded confirming previous experimentation relating to the susceptibility of the oat shoot to infestation, emphasising its limitation to the early leaf stages.

Experimentation concerned with recovery power after injury showed that different varieties of oats react very differently and indicated the importance of the primary shoot in relation to yield. A few field data are recorded to support this inference.

With common English varieties of oats the influence of variety on the rate of growth of the primary shoot was practically negligible, and therefore observed differences between varieties in extents of infestation of primary shoots cannot be ascribed to variations in rates of growth of primary shoots. The time of tillering did not depart markedly from the mean, but it was not correlated with time of shoot production, so that correlation between extent of infestation of shoot and of grain cannot be expected.

Excess of nitrogenous manure failed to influence the extent of shoot infestation but effected a reduction in the extent of grain infestation during a season when the extent of the attack of the fly was below normal.

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OBSERVATIONS ON THE DISEASE OF OATS CAUSED BY THE STEM EELWORM *ANGUILLULINA DIPSACI* (KÜHN, 1857)

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(With 3 Text-figures.)

Introduction.

Although the eelworm disease of oats caused by *Anguillulina dipsaci* (Kühn, 1857) has been known for more than a century, there are many points in the biology of the pest which have not yet been cleared up. It is the purpose of this paper to bring forward some observations on the biology of the nematode obtained during investigations in the eelworm disease of oats in the north of Scotland. The work was carried out in the laboratory of Agricultural Zoology, Marischal College, Aberdeen, under the supervision of Dr Rennie, Lecturer in Agricultural Zoology. I am especially indebted to Dr Rennie for his helpful advice and criticism and to the Carnegie Trust for a Research Scholarship which enabled this work to be carried out.

The Nematode.

The nematode causing eelworm disease of oats was first described by Kühn (1858) as being the cause of a disease—"Kernfäule," in the heads of Fuller's thistle or teasle (*Dipsacus fullonum*). He accordingly named the nematode "*Anguillula dipsaci*." He found afterwards that the same nematode could attack oats and other plants, so he considered the name *Anguillula dipsaci* Kühn 1858 too restricted and therefore changed it to *Anguillula devastatrix* Kühn 1858, which conveyed more meaning.

Bastian (1865), however, showed that nematodes, besides being more numerous in species than was formerly supposed, had widely different structural characters. He proposed to classify *Anguillula dipsaci* Kühn with several other species in a new genus *Tylenchus*, a classification accepted by most zoologists.

Prillieux (1881) investigated the eelworm disease of hyacinths, naming the nematode *Tylenchus hyacinthus*, while two years later Beyerinck published a paper on the eelworm disease of onions which he attributed

to *Tylenchus allii*. Bos (1888), definitely proved that *Tylenchus hyacinthus* Prillieux 1881, *Tylenchus allii* Beyerinck 1883, and *Tylenchus devastatrix* Kühn were one and the same species and he gave a list of some forty plants susceptible to the attacks of this nematode.

In a recent publication Bos (1922) has added to the number of host plants of *Anguillulina dipsaci* which now number 67.

Baylis and Daubney (1926) revive the old generic name *Anguillulina* Gervais and van Beneden 1859, treating *Tylenchus* Bastian (1865) as a synonym. The name *Anguillulina* is justified by the laws of zoological nomenclature. According to Baylis and Daubney therefore the correct generic name of this nematode is *Anguillulina dipsaci* (Kühn, 1857). The synonomy and classification is given in detail by Baylis and Daubney whose work should be consulted. For a morphological description of the nematode I refer the reader to the works of Ritzema-Bos (1892) and Marcinowski (1909).

Life-history.

Observations show that the parasite may enter the oat seedling as soon as the sixteenth day after germination when the first true leaf has emerged 2–4 in. from the coleoptile. The larva or adult nematode takes up a position in the parenchyma of the sheathing leaf near the growing point. Several nematodes may enter a single seedling. Eggs are laid very soon which have an incubation period of 5–7 days. The young active larvae, which move about freely in the plant tissue, grow and moult a number of times before they become sexually mature in 22–28 days. Goodey (1922) found by inoculating clover seedlings showing the first true leaf with a single egg containing a well developed larva, that mature and sexually differentiated worms were developed in 24–30 days from the date of inoculation. On account of this comparatively rapid development from two to three generations of *A. dipsaci* may be produced within the year, until the plant presents conditions no longer favourable to the nematode's development.

Plants heavily infected in the early stages of their growth die and decay into the soil. Others continue to live but make little headway in growth. The toxicity associated with the decay of infected plants is accompanied by migration of the eelworms into fresh portions of the plant or into the soil. Frequently migration is difficult and impossible. Eelworms in fresh parts of the plant may be prevented from migrating to the soil owing to the advanced state of decay of the base of the plant. Again, the plant may dry up so quickly that migration is impossible.

In such circumstances the eelworms become quiescent. In this state they can persist in the case of worms from narcissus for a period of 2¼ years (Goodey, 1923).

Towards maturity of the oats the majority of the eelworms will have migrated into the soil. So far no one has attempted to estimate the number of *A. dipsaci* in the soil after harvest and before seeding. The following soil examinations were carried out for the purpose of arriving at the number of *A. dipsaci* in the soil and the depth they are likely to occur. The samples were taken from an area of 1 sq. yd. in the "eelworm" infected field at Mains of Dunottar, Stonehaven. This field is of heavy wet clay and the oats in 1926 were badly infected. The numbers are estimated from ½ cu. in. samples.

Result of examinations of soil samples from "eelworm" infected field, Mains of Dunottar, Stonehaven.

½ cu. in. estimates.

Date of lifting the sample	Depth of sample (in.)	Total no. of *A. dipsaci*	Males	Females	Larvae
22. ix. 26	0–2½	473	9	18	446
22. x. 26	0–½	118	1	3	114
	2½–3	106	—	—	106
	5½–6	1	—	—	1 (dead)
22. xi. 26	0–½	215	3	7	205
22. i. 27	0–½	96	4	8	84
22. ii. 27	0–½	58	2	4	52*
13. iii. 27	0–½	92	5	12	75†

* Five of the larvae were sexually mature but still within their last moult.

† Thirteen of the larvae were sexually mature but still within their last moult.

These examinations show the enormous numbers of *A. dipsaci* that may be present in infested soils. An important point arises regarding the results obtained for February and March. Here it will be observed that 5 and 13 of the larvae were found in their last moult before becoming mature adults. This seems to indicate that a certain amount of development takes place in the soil during the warmer months of February and March since no pre-adult larvae were found sexually mature and in their last moult in any of the soil samples examined previous to February. I consider then that a percentage of the pre-adult larvae migrating from infected plants into the soil at harvest can undergo the last moulting stage in their development to become adults. This partly explains the finding of adults in oat seedlings in April. The soil in which the seed has been sown in March contains a considerable number of recently matured adults, capable of entering the oat seedling.

From the soil examinations it is seen that it is the larval stage of *A. dipsaci* that is mostly present in the soil. The intestine is observed to be fully packed with food material. These larvae are the same forms observed in the diseased plant before migration to the soil and are undoubtedly the forms which can persist and withstand longest the changeable conditions of environment from one year to the other. In regard to these worms in narcissus Goodey (1923) says, "It is possible to differentiate males and females by the general shape of the tail region, and by the fact that the situation of the vulva is distinctly marked in the females...they seem, in fact, to pass into the quiescent condition well stored with reserve food granules with the internal anatomy in that stage of transition which just precedes the final complete differentiation."

External symptoms of diseased plants.

A feature of eelworm disease in oats is that infected plants may be beyond recovery when only slight symptoms of disease are apparent. Germination in every case takes place normally and it is not until 6–7 weeks later that an abnormality of growth is seen in attacked plants. Without exception the young plants appear quite healthy, tillering freely. Many of the plants at this stage, however, if uprooted and examined closely will show distinct swelling above the first node, the leaves being normal in formation and colour. The symptoms of disease in seedlings germinated near the surface of the ground are very different, and it is their characteristic appearance which leads one to suspect eelworm infection in a crop in the early stages of its growth. Here the leaves are very broad and bright green in colour. They appear stiff and rigid and are slightly thicker to the touch than those of healthy plants. From 1–2 weeks later such a plant invariably produces an abnormal number of tillers which give it a decided bushy appearance. No paleness of the leaves is yet observable, but they are very liable to rust infection. True roots are sparsely formed and this accounts for a large percentage of the dead plants found in a diseased crop at this stage.

The first six weeks after germination may be conveniently termed Stage I of the disease symptoms characterised by:

(i) Increased formation of tillers and early mortality of plants growing from seed germinated near the surface of the ground.

(ii) Swelling and thickening of the stems in plants germinated at least $1\frac{1}{2}$ in. below the surface of the soil.

About three weeks later a very great difference in the appearance of infected and non-infected plants is observable. While healthy plants

may have reached a height of 9–11 in., infected plants remain very stunted. This can be seen from the following measurements of 12 diseased and 12 healthy plants taken at random (measurements taken from the base of the plant to the tip of the longest leaf).

Diseased	Healthy
2·3 in.	7·5 in.
3·5	8·5
3·5	8·7
5·0	8·8
5·0	9·0
5·2	9·5
5·3	10·0
5·5	10·1
5·5	10·2
6·0	10·4
6·1	10·6
7·5	10·7

Of importance also is the increase in number of tillers in diseased plants.

Count of tillers in diseased and healthy plants.

Diseased	Healthy
5	1
5	1
3	1
3	0
3	1
4	1

The tillers do not develop normally; they are markedly swollen and distorted. Several never appear above ground and are twisted down into the soil. In this state they remain white or cream coloured, growing sometimes to a large size. At this stage very little abnormality in the leaves is observed; infected plants often appearing very luxuriant with bright bluish green leaves. In many cases the leaves are twisted in a spiral fashion, frequently showing waviness. The leaves of the plant at the junction of the roots and stem continue to enlarge giving the plant a "tulip root" appearance, while the root system may entirely disappear. Brown areas are already present in the leaves surrounding the base of the plant.

From the sixth to ninth week after germination may be taken as the second stage in the symptoms of the disease characterised by:

(i) Poor development of the internodes causing the plant to remain stunted.

(ii) Continued formation of tillers which, instead of growing erect and bursting into leaf, remain pale in colour, dwarfed and contorted.

(iii) Twisting and crinkling of the leaf blade.

(iv) Browning in the leaves at the swollen base of the stem.

Few new features in the symptoms are to be observed until three weeks later when a decided contrast between infected and non-infected areas is found. Infected plants have now lost their green colour, the leaves looking pale and sickly. This chlorosis is often marked in badly infected plants. Diseased tillers break off easily from the plant through the basal tissue being in a state of collapse and decay. This occurs also in those infested plants which have succeeded in maintaining their root system under heavy infection. One finds it comparatively easy to pull up such plants leaving the roots in the ground owing to the damage and weakness of the tissue at the junction of the roots and stem. In many cases the root system entirely disappears. Such plants, spongy and dried up in appearance, are light in weight. The broad, stiff and erect leaves present a marked contrast to the graceful drooping habit observed in the leaves of healthy plants.

From the ninth to fourteenth week after germination marks the third stage, bearing the following characteristics:

(i) Chlorosis of the leaves.

(ii) Decay of the tissue at the base of the tillers and the stem.

(iii) Thickening and broadening of the leaves which stand rigid and pointed upwards in a characteristic manner.

The fourth and final stage before harvesting (sixteenth to nineteenth week after germination).

Diseased areas are easily observed in a field at this stage on account of the scarcity of plants and the green condition of those still surviving the infection. Those plants which have succumbed to the disease have almost disappeared off the ground. Many stunted plants are to be found with a well-developed and almost ripe panicle of oats within the sheathing leaves. Fig. 1 is typical of the plants referred to. This plant was 14 in. high, stunted, with poorly developed internodes. The ear, partially emerged from the sheathing leaves contained 26 grains. Six of the grains were dwarfed and pale green in colour, the rest being of good size, ripe, and well formed. The nodes were very dark-brown in colour and dry, and crumbled readily into a powder when subjected to pressure. A small portion of the powdery material moistened with water on a slide revealed



Fig. 1.



Fig. 2.



Fig. 3.

Fig. 1. A ripe panicle of oats infected with eelworm in the rachis and grains. Note the short decayed internodes at the base of the panicle.

Fig. 2. A badly diseased unripe panicle of oats with several malformed and infertile grains. The black shaded areas shown in the glumes and elsewhere are dark brown in the living plant and are due to the presence of eelworm.

Fig. 3. Oat panicle infected with eelworm. Note the infertile grains and the twisted and swollen nature of the rachis.

many *A. dipsaci* in a quiescent state. All stages of development of eelworm were observed, eggs, larvae and sexually mature adults. Many of the adults were in a shrivelled condition and dead. The last stage larvae had nearly all assumed a coiled condition. On contact with the water the larva gradually uncoiled. After 20 min. independent movements commenced. In 45 min. both larvae and adults were moving about freely in the water.

To test whether the seed was infected or not, six grains were detached from the rachis and examined in water. In the first seed examined from the inner face of the pales two larval *A. dipsaci* floated off. One was already active, the other soon moved freely. The following is the result of examination of the six grains:

Result of examination of six grains for A. dipsaci. *September 2nd.*

Grains	No. of *A. dipsaci* per grain	Remarks
1	2	1 active and 1 in a quiescent state
2	1	Quiescent
3	2	Quiescent
4	0	—
5	3	Quiescent; 1 shrivelled and dead
6	0	—

Careful examination of the stem and the rachis carrying the grains revealed in these signs of nematode infestation. In three cases the rachis was decayed in the centre and in one, two larval *A. dipsaci* were found actually at the junction of the rachilla and rachis.

Many plants similar to the above but in a green condition could be gathered from infested areas. A typical specimen of these plants is seen in Fig. 2. Again in this case only three grains appeared from the surrounding leaves. These were taken off in order to show the malformed panicle to better advantage. A feature to be observed in this plant is the swollen and twisted nature of the stem. Several aborted flowers, consisting of shrivelled glumes, can be seen at the lower part of the stem. Dark brown patches occur in parts of the glumes surrounding the poorly developed seed. On closer examination this was found to be entirely due to the presence of *A. dipsaci*, which were still active in the tissue of the swollen glumes. The stem and rachis were badly infected which accounts for their distorted appearance.

Fig. 3 is another example of infection of the panicle showing how greatly it may become reduced in length. This panicle consisted of three

large and three small grains partly emerged from the sheathing leaf and in a half-ripe condition. The following is the result of examination of the grains:

Result of examination of six grains from an infected panicle. September 7th, 1926.

Grains	No. of *A. dipsaci* between the glumes and pales	No. of *A. dipsaci* between pales and kernel	Remarks
1	0	1	Female active
2	0	0	—
3	15	6	1 male; 3 females; 10 larvae
4	2	3	Larvae
5	1	2	Larvae
6	4	1	Larvae

Both adults and larvae were also found in the stem and rachis.

The latter observations and examinations are indeed very important as they reveal hitherto unknown facts in the biology of *A. dipsaci* on oats. Ritzema Bos (1888) in his criticism of the remedies for nematode disease in rye makes the following statement: "Il a été demontré longtemps avant moi, notamment par Kühn et Havenstein, que l'incinération des graines provenant d'un champ infesté est non seulement extrêmement onéreuse, mais de plus totalement inutile, puisque jamais il ne se trouve dans les graines de seigle des *Tylenchus* de l'espèce qui nous occupe. . . les anguillules de la tige (*Tylenchus devastatrix*) restent toujours à l'intérieur des tiges et des feuilles, sans jamais se rendre dans les fleurs ni dans les graines." Marcinowski (1909), with reference to the biology of *Tylenchus dipsaci*, says, "Er steigt nie in beträchliche Höhe hinauf, ist in Hafenispen noch nie gefunden worden, nur in ganz seltenen Fällen in der Achse der Ähren, die aber aufstehend, nicht hoch gewachsen waren."

Hodson (1926) in the course of examination of a large number of seeds from badly infected fields observed occasional occurrence of quiescent *A. dipsaci* larvae beneath the pales of the seed; continuing he says: "While the number of cases found is so small as to suggest that the occurrence is accidental, careful watch should be kept for any development in this direction, as it has been found recently in America (Godfrey (1924)) that seed dissemination is the normal mode of spread adopted by this eelworm when occurring on certain Compositae." Hodson was therefore suspicious of *A. dipsaci* being capable of infecting oat seed.

The results of my observations prove beyond doubt that *A. dipsaci* is able to enter the inflorescence, to live there and to cause infection of

the grain. This infection is not merely occasional, but is found very frequently in stunted plants which have succeeded in developing an ear under heavy infection of the parasite. In a diseased area of 36 sq. yds. in the infected field at Mains of Dunottar farm I obtained no fewer than eleven infected panicles, and five of these had the grains partly emerged from the sheathing leaves. A number of the panicles were stored in the laboratory to allow the seeds to dry and become firm. The grains were threshed out of the glumes three months later and examined as before by tearing the pales apart. Thirty-seven seeds were examined in all, 24 of these being infected, giving approximately 65 per cent. infection.

Without doubt these stunted plants, which varied from 10–14 in. in height, would have been cut and harvested with the healthy grain. Particular attention was thus paid to the harvesting and stacking of this infected field which was threshed out in December. A sample of the grain had a better appearance than one would have expected. Fifty of the light and less well-developed grains from a sample were examined; four of these gave the following number of *A. dipsaci* in a quiescent condition beneath the pales:

(1) Three larvae—one shrivelled and dead.

(2) Five larvae—revived in 25 min.

(3) Two larvae—one shrivelled and dead.

(4) One larva—revived in 27 min.

It occurred to Hodson (1926), who is probably the first to establish *A. dipsaci* beneath the pales of oat seed, that the larvae had arrived there accidentally. This notion need not be entertained further since, as I have shown, *A. dipsaci* can infect the panicle and the developing grains.

Unfortunately there is nothing to distinguish infected and non-infected oat grains. It is impossible therefore to recognise a sample of oats from an eelworm infected area. True, there are many poorly filled and green tipped seeds to be found in a sample of eelworm infected oats, but these may be due to other factors such as poor soil, improper manuring or adverse climatic conditions.

Hodson (1926) has already shown that infected seed may produce infected seedlings.

It can be readily understood that definite proof of infection of the seed is of the greatest economic importance. Up to the present it has been generally accepted that a farmer was quite safe in sowing seed from an infected area, on the ground that eelworm never entered the seed. In future farmers would do well to pay more attention to the

source of their seed if they wish to avoid infection of their soil and crops. Spread of infection by the seed may contribute largely to the explanation of presence of the disease in hitherto uninfected soils.

SUMMARY.

1. The life-history of the nematode is described.
2. The numbers of eelworms occurring in infected soils is discussed.
3. The external symptoms of diseased oat plants are described in detail.
4. It has been definitely proved that *A. dipsaci* can infect the oat panicle and grains and remain in a quiescent state within the harvested seed.

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PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

ORDINARY MEETING of the Association held at 2.30 p.m. on March 23rd, at the Imperial College of Science. The President, Dr E. J. BUTLER, C.I.E., F.R.S., in the Chair.

Subject: "Forest Products Research."

(1) "Research in Progress at the Laboratory at Princes Risborough," by R. S. PEARSON, Esq., C.I.E., Director of the Forest Products Research Laboratory.

(2) "The Work of the Oxford Branch of the Forest Products Research Laboratory," by J. F. MARTLEY, Esq., M.Sc., A.R.C.S.

(3) "The Work of the Mycological Section of the Forest Products Research Laboratory," by K. ST G. CARTWRIGHT.

(1) MR PEARSON.

THE speaker opened his address by describing the position which Forest Products Research took with relation to a complete Forest Laboratory, and as an example instanced the organisation of the Forest Research Institute, Dehra Dun, India.

He explained that in the case of a complete Forest Research Institute, branches of Entomology and Botany would form part of the organisation, and that therefore the Economic Branch which deals with Forest Products Research would consult the other branches for assistance in connection with Mycological and Entomological problems.

In the case of a Forest Products Research Laboratory being an organisation by itself, it was necessary to form sections of Entomology and Mycology within the Forest Products Research organisation. Speaking on the subject of Forest Research Institutes, he pointed out the danger of splitting up the various branches dealing with Silviculture, Botany, Chemistry and Utilisation, and having them located in different places, and cited the case of America where all the former sections were situated at Washington, while the Utilisation Research Station was at Madison.

Having described the general organisation of Forest Research, he continued his address by describing the internal organisation of a Forest Products Laboratory. The lecturer pointed out that the functions of such a laboratory were primarily to determine more economic methods of dealing with various forest products, to find new uses for the more uncommon timbers and to assist users of timber by advising on their many problems connected with the use of timber.

The speaker continued by describing the activities of each individual section, giving examples of the type of work carried out.

The work of the Wood Technologist was first described, followed by that of the Section of Seasoning.

The work of the Timber Mechanics' Section was next dealt with, and it was pointed out that the investigations fell into three classes, namely, routine testing—to determine the relative strength of different timbers, structural tests—the strength of timbers of different grades and different species, and special tests—such as might be carried out on wear of paving blocks, strength of tool handles, strength of gun-carriage wheels, etc.

The work of the Wood Preservation Section was next dealt with, and examples given of investigations carried out in India on sleeper woods, and the difficulty of determining the toxicity of various antiseptics.

A brief mention was made of the work now being carried out at Princes Risborough by the Entomologist, and by the Mycologist working at the Imperial College of Science and Technology.

The speaker ended with a brief reference to the very extensive field covered by Minor Forest Products, and an instance was given of the work carried out by the Economic Branch of the Research Institutes in India, on bamboo for paper pulp.

(2) Mr Martley.

The speaker said: It is now some years since the Forest Products Research Laboratory was started, but, because those who were mainly concerned in its establishment were desirous of it being started on sound lines, little has been heard of it until recently. This time has been devoted to the work of delimiting the field of investigation, to the study of those problems which are likely to arise and to the cultivation of what I might call a sense of forest products research. In this phase of the work we have had the inestimable advantage of tempering our own initiative with the experience gained by other countries where forest products research has already been established for a considerable period.

Though we are still far from the moment when we can point to the first fruits of this preliminary work, yet the description of the problems that are met with in forest products research and the methods with which we propose to attack them should be of great interest to you all. And this because in the last analysis they call for economic solutions, and because in the earlier stages a wide experience of scientific knowledge is needed to guide the investigators through a difficult field of endeavour.

At this point I should like to quote the opinion of a famous astronomer on the subject of forest products research which cheers me up whenever I am overcome by my own ignorance of wood, and its many properties. Prof. Eddington, as a foil to his own achievements in unravelling the story of the birth of the stars says: "To understand all that is going on in the material of a piece of wood is a really difficult matter, almost beyond the aspirations of present day science."

Our Director has just been describing to you the development of forest products research in India and other countries, its recent establishment in Great Britain and the work that is now being carried out at Princes Risborough under his direction and guiding influence.

It now falls to my lot to describe the work that is being done by a section of the Laboratory that has been partly nurtured in its earlier stages under the wing of an ancient and honourable seat of learning.

In describing the work that is being done at Oxford it is not always easy to distinguish between the work of our own laboratory and that of the Imperial Forestry Institute. As our respective problems are often closely interwoven a system of close co-operation has developed between the two institutions. I shall therefore treat of the work as a whole and will not refer further to their respective shares in the work beyond mentioning that the Imperial Forestry Institute is in closer touch with those problems connected with the living tree, while we on the other hand are dealing with the product of the forester's skill.

The first necessity in any problem is to know your material. In our work this first essential is dealt with by the Imperial Forestry Institute who have developed a scheme whereby the forest services of the different Dominions and Colonies send home samples of their commercial timbers together with botanical material from the same tree as a check to the botanical identification.

A collection of several thousand identified woods has already been built up, to which additions are constantly being made as fresh material comes to hand. From this collection type slides are cut for the investigation of the structural features of the wood.

The use of this collection is twofold. The first is purely practical: it is the guide that has to be employed when an unknown timber has been received for identification. In general it is possible to give a satisfactory commercial identification, but from the botanical side it is often impossible to do more than run down a specimen to a group within a genus, as for example in the case of the Pines and Oaks.

In the second case the systematic study of the collection is bound to raise many points of scientific interest. Up to date little attention has been paid to the structure of wood from a systematic point of view which is somewhat surprising considering the amount of work that has been carried out in recent years on the detailed structure of the timbers of the world. Even Moll and Janssonius, in their monumental work on the structure of the woods of Java, do not attempt to correlate structural features of wood with those features of proved systematic value which are the key to a classification based on evolution.

Prof. Jeffrey has laid down on *a priori* grounds an evolutionary sequence for certain types of structure notably in regard to the distribution of medullary ray tissue and of wood parenchyma, but with the exception of the gymnosperms there has been little attempt at correlation between the study of evolutionary sequences in wood structure and those that are of importance in systematy.

I will now go on to the description of another phase of the study of wood structure which is not only of great scientific interest but which is absolutely essential in any complete study of many forest products research problems.

As I mentioned before, you must know your material. In wood there are as many types of material as there are species. In any one species it is found that within certain limits, which are of a specific nature, there are large variations in both the grosser and more minute structural features of wood.

To settle economic problems which are connected with the variation in structure of a particular wood species it is absolutely essential that the normal extent of these variations should be determined not only within a particular tree but also in relation to climatic, environmental and silvicultural conditions.

At present we are starting on such an investigation of the structure of three species native to Great Britain, viz. Oak, Ash and Elm.

The general procedure in this investigation is to select suitable material from the merchantable portion of the trunk of trees that have been identified botanically. The material chosen consists of at least three discs, the number depending on the length of the bole, one being taken from near the base, one from below the crown and the others from intermediate positions.

Microscopic sections prepared from these discs are examined for variable features and these features are related to age and to position in the tree. The following are some of the features that have been found to be of interest in this study: gross features which include density, percentage spring wood, percentage summer wood, ring width and finer features which include size of pores, wall thickness, fibre length, percentage of fibre (as area on transverse section) and ray volume (as area on tangential section).

Once a standard has been established for any given wood or variety, the necessary background has been obtained by which correlation of structure with other properties can be effectively attained.

These properties can be classified under three main headings: Mechanical, Seasoning and Utilisation.

The method of correlation in each case follows the same general broad outlines.

One of the objects of the investigation of the mechanical properties of English grown timbers is to establish the normal strength variation that is to be expected in commercial timber.

Material for this purpose is obtained from specially selected and botanically identified trees, and the normal strength variation is determined not only within a given tree but also its variations from locality to locality. At the same time the relation of the strength values to the grosser structural features such as density, ring width, and percentage of summer wood is worked out.

In many cases, however, it is apparent that a deeper investigation into the structure is required in order to explain apparent anomalies. This can only be done satisfactorily when both the structural and strength variations for a given species have been studied in conjunction. After mechanical tests, suitable samples, taken from the test pieces, are examined for those variable features of which a list has already been given.

Although we have not had time to do more than start off on this particular investigation some of the results obtained are very encouraging and in the case of Oak we have been able to confirm the findings of a somewhat similar investigation by the U.S. Forest Products Laboratory at Madison on the brashness of timber, which is a special type of weakness.

In general the rule holds that the strength of a piece of wood is proportional to its density. In Oak, however, a certain proportion of contradictory examples were found, in that some test pieces had a far lower strength value than was to be expected from their density. On investigation it was found, other things being equal, that the strength of Oak was proportional to the amount of fibre present as measured on a transverse section. This fibre tissue is much denser than the rest of the wood averaging about 1·0, whereas the density of the wood as a whole is about 0·65 to 0·75. In the anomalous specimens the low fibre content was balanced by a high proportion of compound rays which also are characterised by a high density. Due to their orientation the medullary rays do not contribute to the strength in the same proportion as they do to the density of the wood.

If time permitted I could give further examples showing how a knowledge of the structural detail is of value in questions relating to the strength of wood.

In the correlation of the structural features of wood with its seasoning properties the procedure is similar to that employed in the case of the mechanical properties. The study of the correlation is however considerably simplified as it is the grosser structural features that will have the greatest influence on the quality of the seasoned article, since the more important seasoning defects are connected with such features as the proportion of spring and summer wood, regularity of growth, direction of grain (straight or cross-grained), presence and size of knots, size and number of medullary rays. The majority of these features are such that their study does not in general necessitate a structural examination of the material by microscope.

The third direction in which the structure of wood is of importance has been given the general heading of Utilisation. This includes all problems where the particular structure of a wood has a decided bearing on the use to which it is applied.

In the time at my disposal I can do no more than give a few indications of the work that comes under this head.

One of the lines of investigation that the Laboratory has in hand is the working and machining qualities of the different commercial woods. In connection with this investigation the variation in the structure of oak is being studied, and from this study it is seen that there is a very close relation between the material preferred for cabinet making and the finer structural details.

This investigation, comparatively simple in itself, leads up to problems and gives suggestions as to their solution which are far beyond its original scope. And it is on account of reasons such as this that the problems met with in forest products research are of such fascinating interest.

In the case of oak it is found that certain structural features are characteristic of the material which is preferred for cabinet work. It is also known that material from certain localities is much sought after and is chosen in preference to any other even from the same neighbourhood.

It is expected that the outcome of this investigation will determine to what extent this preference is due to the particular variety or form (*e.g. Q. Robur, Q. sessiliflora* or hybrid) and how much is due to soil, climate, silvicultural conditions, prejudice and the hundred and one other factors that enter into all economic questions of supply and demand.

The Laboratory, however, has to leave the problem at this point as its further pursuit belongs to the domain of the forester or forest botanist, that of our sister institution the Imperial Forestry Institute.

One side is the elucidation of the factors which, during the growth of the tree, dispose the wood toward one or another of the extremes of structure which is normal for the tree species under the general range of conditions found in nature. The second half of the forester's problem is so to study his silvicultural methods as to produce timber of the quality which is required by the timber user. The second half of the problem is more complicated than it might appear at first sight, for economic factors have to be taken into account in the case of timber-growing which apply 100, or even 200 years hence when the timber is coming to maturity; no mean task.

We now leave the work that centres round the structure of wood. Although up to the present no very startling facts have been discovered, the preliminary work of

exploring the field of endeavour has been roughly completed; but I hope that within a few years information of value will be acquired.

The next series of problems with which I will deal centre round the decay of wood. I will not be able to tell you much on this particular subject because as far as we are concerned the preliminary work of investigation has not been completed.

The position up to date is that decay is caused by fungi and insects, and that by soaking or injecting wood with chemicals, poisonous to decay organisms, their ravages can be warded off to the extent of making wood preservation of economic importance, and this in spite of the fact that the present methods of wood preservation are both expensive and to a large extent wasteful of material. As the possibilities of this method of attacking decay are reaching or have reached their limit, it is the intention of this laboratory to initiate an attack on the problems of decay from another vantage point. At the present moment it is of course impossible to say whether results of economic importance will be obtained, but, as in many other walks in life, nothing ventured nothing done.

It has taken the last ten to twenty years to develop a technique by which chemical analyses of wood can be carried out so as to give accurately reproducible results, and latterly considerable attention has been paid to the decay of wood in the light of this work.

In passing I should like to mention that an interesting outcome of this work has been to show that the mycologist's classification of wood-destroying fungi into white rots and brown rots is purely descriptive and is no indication whatever as to whether the cellulose is attacked at the expense of the lignin or *vice versa*.

In this work on the analysis of decayed wood no attention has been paid to what happens in the earlier stages of decay and little to the mechanism by which decay is brought about.

It is hoped that by studying the chemistry of decay in the early stages it will be possible to find out the first effect of a wood-destroying fungus on wood, and then with this fact in hand to deduce a method which will be effective in preventing the initial stages. This method, if it proves successful in tackling the question of decay, resembles the method employed by the belligerents during the late war. Its spirit will be more in keeping with the Hague conventions as it will mean a cessation of "frightfulness" methods and only entail a mere starvation of the fungus into submission.

There is another side to the chemical study of wood, which is only coming into prominence again due to the recent advances in the methods of the chemical analysis of wood.

The last work of importance dealing with the building up of the cell-wall of woody plants dates from the eighties of the last century, when the detailed structure was investigated and correlated with the then existing state of knowledge of the chemistry of the cell-wall. During this period the botanists investigating the problem had the then existing knowledge of the chemistry of wood and of cellulose at their finger ends and, if not directly working on the chemical side of the subject themselves, had an important share in its direction and control.

Since that date the botanist has not furthered to any appreciable extent this investigation into the detailed structure of the cell-wall, and his chemical conceptions have not advanced much beyond the limits reached by the past generation of botanists as can be judged by references to the current text-books on Botany.

The chemists, both on the continent and especially in America, have, independent of the botanist, made considerable advances into the chemistry of cellulose and wood of which botanists, judging from their literature are not sufficiently aware.

The time has been reached when both the botanist requires the help of the chemist and the chemist that of the botanist in order to initiate a new attack on the growth and progressive changes, both chemical and physical, which occur during the development of the lignified membrane in woody tissue.

At the present moment there is unlimited confusion in the literature on the debatable territory lying between the chemist and the botanist which is enhanced by the misuse of terms which are in common use by both parties to the discussion.

A pioneer attempt to clear this encumbered field for action has been initiated by the chemists working at the Forest Products Laboratory at Madison, who have shown definitely that it is possible to separate two distinct types of "lignin" from wood. One type is derived from the middle lamella, which term is apparently used to cover the middle lamella proper and the primary wall, and the other from the secondary layer. This finding is foreshadowed by the work of Sanio on *Pinus sylvestris* where in the development of the cell-wall from the time of cell division in the cambium, he observed that before lignification the chemical nature of the primary and secondary layers was different and that this difference still existed in some form after the process of lignification had been completed.

Work along these particular lines is of real scientific value and interest, but it is not likely that with so much other work in hand we shall with our present staff be able to contribute materially to this particular subject for the present. Yet undoubtedly this study is very necessary and will yield results of practical value in the course of time because it is only investigations of this nature that can supply the necessary basis for constructive criticism of the current methods employed in combating the destructive activities of the various wood-destroying organisms.

There is another side of our activities at Oxford I have not touched yet which deal with the physical properties of wood and which centre round its relation to moisture in all its forms. This is a big subject in itself especially as it ties up so closely with many of the other properties of wood. As time is drawing on I am afraid I shall not be able to treat of more than a few of the many ramifications which the subject of the relation of wood to moisture possess.

One of the most important of the aspects of the moisture relationship of wood is that which bears on the seasoning of wood.

From small-scale experiments the general course of the drying of wood has been followed. As a result it is possible to state that to a first approximation the general law of diffusion applies to the drying of wood. It has been shown that the rate of moisture movement in wood is proportional to the vapour pressure gradient, and that the effect of temperature on moisture movement is due mainly to its effect on the vapour pressure of water. The rate of moisture movement is also dependent on the actual moisture content as well; the movement through wood at the fibre saturation point is about three or four times as fast as in the air dry state for the same condition of the vapour pressure gradient.

Another important aspect of the relation of wood to water is its hygroscopicity. Its importance is due, first, to its relation to moisture movement and drying and, secondly, to the effect of moisture content on dimension.

The behaviour of wood in this respect is closely paralleled by numerous other colloidal substances, for example, gelatine, starch, gums, casein, and soil to mention only a few. Wood itself however has been the subject so far of very few investigations of this or related properties.

To fill this gap an investigation is now being planned, and it is hoped that useful information may be obtained in due course for dealing among other things with that troublesome property of wood of changing its size with the varying moisture content of the surrounding air which is the *bête noire* of the furniture manufacturer who has to explain why the drawers in his furniture have ceased to run after leaving his factory.

This brings to a conclusion my account of the work of the Forest Products Research Laboratory at Oxford, the type of problems we meet and the methods we use to obtain the information needed for their solution.

(3) Mr Cartwright.

The speaker said: Very little work has been done in this country on the study of wood-destroying fungi and this is especially the case in regard to their behaviour in culture. Identification of the fungi causing decay has, in the absence of sporophores, consequently depended on the gross characters of the rot. Though in some cases these may be characteristic, they are often misleading if depended upon alone, and in the early stages rarely afford sufficient basis on which sure identification can be made.

The fungi are usually for convenience divided up into those causing brown rots and those causing white ones. In some cases a fairly accurate diagnosis may be made on such characters, when combined with examination of the type of bore holes, etc., but frequently it is impossible to be sure of the causal organism, especially when the decay is in an early stage. Identification becomes still more difficult when, as often happens, two or more fungi are at work. Actually the presence of fruit bodies is rare and, failing such evidence, the only method is to isolate the fungus or fungi present in the wood and to identify them by means of their cultural characteristics. The importance of this work for rapid diagnosis of the causes of decay has been recognised in America and Canada where type collections have been built up, notably at Madison under the charge of Dr Audrey Richards and at Ottawa by Miss Fritz (1), who has published a paper giving descriptions of a number of these fungi in culture. As no such type collection existed in this country and the collection at Baarn contained very few of the higher Basidiomycetes, it appeared of prime importance that such work should be undertaken at once and the Mycological Section of the Forest Products Research Laboratory has made the building up of such a collection its first object.

It is hoped eventually, with the aid of type specimens of rot, slides showing details of the fungus in the wood, together with these cultures, to keep what would be virtually a reference library for diagnosis of timber decay, so that this side of the work might become part of the routine of the department. This will allow more time to be spent in the study of the physiological relations of the fungus and its host, leading to the solution of problems of such great economic importance as the reason for the varying degrees of durability of tree species.

The more the cultures of these fungi, which mostly belong to the Basidiomycetes, are studied, the more it becomes evident that they exhibit very definite characters

by which they can be distinguished, though at first sight these differences may escape observation, as many cultures appear superficially alike.

Characters on which diagnosis from cultures is found possible are many: a brief list of some of the more important is given below.

The cultures may be divided in the first instance into those showing colour and those uncoloured, when grown on certain standard media. Colour changes during development and the effect of light on the colour range may at times also be found helpful.

Rate of growth in relation to temperature and whether the submerged mycelium grows in advance of the aerial, texture of the mycelial mat and other details of a similar nature. Resistance of the fungus to acidity or alkalinity of the medium—the change in the *p*H value of the medium by the growth of the fungus has not been found of much use as in most cases it reaches a similar degree of acidity (about *p*H 5). In some cases the formation of fruit bodies, normal or abnormal, or of bulbils or sclerotial bodies may assist identification. In many instances diagnosis can be made solely on these outwardly visible characters; but at other times microscopic examination is also necessary. Generally speaking, these fungi are found to have definite microscopic characteristics; some of these which have been found of value are given below.

The range of size of the mycelium both aerial and submerged. The presence or absence of clamp connections. The type of clamps and their distribution. The type of branching of the hyphae. Presence of thick-walled fibres. Lastly, secondary spore formation may take place, sometimes only on certain media. Such secondary spores may be of the chlamydospore type, or oidial in chains, or conidial forms such as the very striking one described by Brefeld (2) under the name *Heterobasidion annosum*, this being a conidial form of *Fomes annosus*.

It may appear from this account that the identification of these fungi by the methods described is rather complicated, but in practice it is rare that more than a few of these characters will be necessary in any one case, and often the identification can be made merely on the colour and general appearance of the culture, without any microscopic examination or physiological test.

In addition to the economic importance of this aid to quick identification, which renders it possible in many cases to discover the place and time of infection, it may help to overcome the difficulties which Systematists encounter in identifying many of the more difficult species, as for example white Poria and Polyporus species. It should also assist in clearing up other problems of nomenclature. It is thought that in all probability many of the species of such genera as Irpex may be found to be merely forms of well-known members of the higher Basidiomycetes. This opinion is based on the fact that already various forms of fructification have been described by Falck (3) for Lenzites sp. and abortive and non-typical forms, which have appeared in cultures grown in this laboratory, would certainly have been placed in different genera had they been found in the field.

It might be thought that these forms would be confined to cultures on artificial media, but there seems to be no evidence in support of this view because, in one single species of Trametes, Exidia, Thelephora, Sparassis, Hydnum, Poria and Daedalea like forms have appeared in the laboratory not only in agar cultures, but also on wood. When one species may produce such varying forms, it appears doubtful whether many of the generic differences have any true significance.

In addition to the formation of a type collection of cultures, an investigation is being carried out in collaboration with the Section of Entomology on the relation between insect and fungus attack on wood and, although this work is in its infancy, already very suggestive results have been obtained.

Work is also being commenced on the testing of wood preservatives under standard conditions, and an investigation on the loss of mechanical strength due to decay is to be undertaken with the help of the wood testing section, and it is hoped that with the aid of the Chemical Section some definite correlation between brittleness due to fungus action and chemical change may be arrived at.

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OBITUARY NOTICES

PROFESSOR ANTONIO BERLESE.

(With Plate XXIII.)

It was with feelings of profound regret that members of the Association learnt of the death of Prof. Berlese, who passed away in Florence on October 24th, 1927, at the age of 64 years. During the past half century the name Berlese has been a familiar one to zoologists and he was universally acknowledged as one of the great leaders in Entomology.

Born at Padua on June 26th, 1863, where he received his early education, he later studied in the Liceo Marco Foscarini, Venice. He early showed a particularly keen interest in natural science, especially insects, and was barely 18 years old when he published a descriptive account of the anatomy of *Gryllus campestris* in the *Atti Soc. Ven. Trent. Sc. nat.* This paper attracted the attention of Prof. G. Canestrini of Padua, which resulted in a close association of Berlese with that eminent zoologist and in the well-known studies of the Acarina on which group Berlese soon became a world-famous authority.

After graduating in Natural Science in 1884 at Padua, Berlese went to the R. Istituto di Studi superiori in Florence and two years later became assistant to Prof. Targioni Tozzetti, who was then director of the entomological station there, an institution founded in 1875. After four years with this famous pioneer in Italy of applied zoology, Berlese went to Portici and in 1900 became Professor of agricultural zoology in the R. Scuola superiore di Agricoltura. Three years later he was recalled to Florence to succeed Prof. Targioni Tozzetti as director of the entomological station, which office he held with such brilliant distinction until his death.

Berlese was a wonderful personality, his unbounded enthusiasm, modest manner and kindly disposition endeared him to all those who came in close contact with him. Philosophical in outlook, his temperament was thoroughly in sympathy with the studies to which he devoted his life. The desire to enquire and investigate was present in his nature to an exceptional degree and he found an outlet for his brilliant gifts in studying the marvellous variety of form and habits of insect life. The facts about insects were to him the material by means of which he could obtain a closer understanding of the laws which govern their behaviour. He was not a collector in the usual sense, and in fact was averse from needless destruction of insect life.

Berlese had extraordinary powers of concentration and methodically and persistently applied himself to the many problems on which he was engaged. A man of wide culture and an amazing breadth of knowledge, together with a highly developed artistic spirit, his conversation was delightfully stimulating. Those who worked with him were impressed by the powerful intellectual force of the man and admired the charming simplicity of manner with which he approached a discussion and infused

into it a spirit of enthusiasm. Students from other countries who were attracted to study in Italy under his guidance learned to love this genial, sympathetic and earnest investigator. The writer recalls many memories of the six months spent in his laboratory during 1919.

He was proud of Italy and loved Florence and felt it a privilege to be a fellow countryman of the great leaders of the past in art and science. He spoke of Redi with a feeling of reverence and dedicated the journal *Redia* in honour of that great biologist.

The oil paintings of certain insects made by him in his leisure time, which adorn the walls of the library of the entomological station in Florence, afford proof of his skill with the brush and his great gifts as an artist. His friend and colleague Prof. Del Guercio enthusiastically says that these beautiful examples merit him the title of the Raphael of Insects.

Berlese withal was eminently practical. His profound knowledge of insects was built up from first-hand observations and he devised many ingenious practical methods to assist him in his experimental work. He found relaxation from more intensive work in painting or in devising apparatus for particular requirements. He built a machine for grinding grooves in microscope slides so that the many hundreds of species of mites which he collected and described could be more satisfactorily mounted. He devised a funnel-like apparatus which enabled small insects and other arthropods to be readily separated from humus, leaf mould and other débris. He made a projection apparatus so that he could readily take photographs of drawings and specimens for illustration purposes. Undoubtedly his adaptability in this practical sense contributed to the success he achieved as an economic entomologist.

As a zoologist the work of Berlese falls into two categories: (*a*) the pure scientific aspect of zoology, especially Entomology, (*b*) the applied aspect, particularly agricultural entomology.

The studies of the Acarina, begun during his early association with Canestrini, were continued until the time of his death and his publications on this group gave him an international reputation as a systematist of exceptional merit. In 1924 he published his *Centuria sesta di Acari nuovi*. The magnificent collection of this group of Arachnids which he personally collected and mounted on slides, is probably the finest in existence and is noteworthy for the many beautiful preparations which it contains. As a systematist Berlese surveyed as a whole any group with which he was dealing. His generic and specific descriptions are full, admirably illustrated and amplified by sound diagnostic keys.

During 1882–1903 four volumes appeared dealing with the Acarina, Myriapoda and Scorpionida of Italy, being illustrated with numerous plates drawn by himself. In 1910 his standard monograph of the Myrientomata was published, illustrated with 17 plates of beautifully executed drawings.

From 1894 onwards several papers appeared on the Coccidae and the observations he made on the anatomy of this group of insects, richly illustrated with drawings remarkable for their accurate draughtsmanship and finish, are of outstanding merit.

Any one of these publications would have sufficed to place Berlese in the front rank, but it is perhaps in the two volumes of *Gli Insetti* that we can appreciate his profound knowledge of the anatomy and morphology of insects and understand the broad conception he had of the vast array of insect life. These two volumes, published


Antonio J. Berlese
1911

in fascicles extending from 1909 to 1927, contain 2200 figs. and 17 plates and a total of 2000 pages of text. The subject matter is rich in fertile suggestions and contains much original work based on the author's own observations, experiments and experiences. They form a fitting monument to his ability and industry and signify his devotion to the study of insects.

Apart from these major contributions to science Berlese published freely on many branches of entomology. He was a versatile writer as is shown by the fact that during the years 1880–1927 no less than 238 publications appeared under his name, many of which deal so thoroughly with the subject in hand that they may be considered as monographs. When occasion demanded it he used a bold polemical style.

As an applied entomologist Berlese realised fully the difficulties surrounding the problems of agricultural zoology. He accepted the teaching of his illustrious predecessor Prof. Targioni Tozzetti, that while mechanical and other methods must be developed as an aid to combating insect pests, attention must be given to the safeguarding and diffusion of parasites and other beneficial insects. In this latter respect Berlese achieved remarkable success with the chalcidoid parasite *Prospaltella berlesi* against the mulberry scale *Diaspis pentagona*, which was first established in 1906. The establishment of *Prospaltella* as an efficient means of controlling the mulberry scale was the means of saving the cultivation of the mulberry and thereby the silk industry, not only in Italy but in the neighbouring countries, and its value to these countries can hardly be estimated in money. This example is one of the classic instances of the value of the biological control of insect pests and assures Berlese a place in the front rank in the history of applied entomology.

It is important to note that the discovery and success of *Prospaltella* was not fortuitous but resulted from the application of an idea which was perseveringly followed up and established by the systematic collection of data. In an interesting paper in *Redia* of 1907 Berlese discusses the general question of what we now call Biological control of insects.

Extending over many years, he carried out a great deal of work on the control of the olive fly, *Daucus oleae*. His numerous publications on this difficult problem show the persistent and methodical manner in which many practical measures were tested out, with successful results. He was also directly concerned with the problem of controlling the house fly and devised practical measures which were highly successful.

On the applied aspect of agricultural zoology he had a happy faculty of appreciating fully the practical difficulties in the field which gained him the confidence of the growers. For instance, in the latter part of 1919 he was requested to deal with the invasion of field mice in the Veneto area and stayed for some time personally supervising the campaign on the spot.

In consideration of his valuable services to agriculture, orders were conferred on him by his own country, France and Greece and the outstanding merit of his contributions to the science of entomology were recognised by his being elected honorary member of the Entomological societies of America and most European countries. In 1914 he was elected Honorary Member of the Association of Economic Biologists.

James Davidson.

SIR ARTHUR SHIPLEY, G.B.E., F.R.S.

Master of Christ's College, Cambridge.

The death of Sir Arthur Shipley last September, at the comparatively early age of 66, came as a painful shock to his numerous friends, admirers and pupils, amongst whom the writer is proud to reckon himself. Although he had been in failing health for some time, he had on several occasions rallied with such surprising vigour that hope for his recovery was entertained until the very last.

Shipley was born at Datchet in Berkshire—his father was a brewer, a circumstance which may partly account for his interest in the economic applications of zoology. Like so many of the earlier students of zoology Shipley began his education with a view of becoming a doctor and passed from University College School to St Bartholomew's Hospital; but when he decided to go to Cambridge, he came under the magnetic influence of Michael Foster and F. M. Balfour, and to use his own words "burned his boats" and devoted his life to the study of zoology. After Balfour's tragic death in 1881 Shipley, along with his life-long friend Harmer (now Sir Sidney), was enlisted by Adam Sedgwick, Balfour's favourite pupil, in the enterprise of carrying on the School of Evolutionary Morphology and Comparative Embryology which Balfour had founded, and it was as demonstrator of Comparative Anatomy in that school in the autumn of 1888 that the reviewer first made his acquaintance, when he entered Cambridge as an undergraduate. Shipley was then known to the irreverent youth as "The Blackbird" or "The Robin Redbreast." He was comparatively short and inclined to be corpulent (against which he fought all his life). He had black hair, sparkling brown eyes, a round cherubic countenance, and bright red cheeks. His whole countenance was expressive of kindness and good-humour. He had already made his mark in the zoological world by his researches on the development of the Lamprey and had been elected Fellow and Lecturer of his College. As a part of his duties a year or so later he gave a course in Advanced Zoology which the reviewer had the good fortune to attend. In these lectures he dealt chiefly with obscure groups of "worms" which were much neglected by other zoologists and with the various groups of Arthropoda: he insisted on an accurate knowledge of anatomical structure and whilst giving the current theories on the evolution of the groups he expressed a healthy scepticism as to their validity, a scepticism which was a most useful mental discipline for his hearers in days when students of zoology were still dazzled by Darwin, Haeckel and Weismann. When he resigned his demonstratorship he was appointed Reader in Zoology and became Tutor to his College. He had already published a *Textbook of Invertebrate Zoology* which at that time was one of the most valuable services to the science which he could have rendered. It was a compact book of moderate size with the essential facts to be remembered by the student duly classified and clearly and briefly stated, whereas the other books available to the student were crammed with vast masses of indigestible details. It was at this time that he invited the reviewer to collaborate with him in producing on similar lines a book which should embrace the whole field of zoology, a task which was eventually accomplished in 1901.

Shipley's period of office as tutor was a very great success. His attitude towards his pupils could only be described as motherly. Partly this feeling and partly his

own love of his beloved subject led him to seek to develop in every way the economic applications of zoology so that more careers should be opened to young students of this science. He had a great deal to do with the appointment of Dr Nuttall as Quick Professor of Economic Zoology in 1906. During this period Shipley's kindness and helpfulness had advanced him to a high position in the University. On one occasion only the present reviewer attended a meeting of the Board of Studies in Biology in Cambridge University. On that occasion a violent dispute broke out between the veteran Professors of Zoology and Geology which culminated in most unparliamentary language. The situation was saved by the tact of Shipley who discovered the *via media* by which the dispute was temporarily smoothed over. Beginning as Secretary of the Museums and Lecture-rooms Syndicate in 1897 he became about 1904 a member of the supreme governing body, the Council of the Senate, and was generally regarded as a leader of the Liberal party in University affairs.

Another most valuable service rendered by Shipley was the part which he took in the establishment of the "University Appointments Board." This beneficent institution, a kind of registry office for Cambridge men, has started a large number of young Cambridge graduates, both scientific and literary students, in useful careers. In 1910, amidst the general rejoicing of scientific Cambridge, Shipley was elected Master of his College. As he was unmarried it might almost be said that thereafter the College was cherished as his only child. At his own expense he entirely renovated the Master's Lodge, transforming it from a somewhat decayed building into one of the most beautiful lodges in Cambridge. He entertained lavishly, and when the war broke out in 1914 he strained every resource to entertain and help wounded officers in his Lodge. During the worst submarine period in 1917 he crossed the Atlantic on a Government mission and helped in bringing about co-operation with America. He understood the Americans well and had many friends there, and he was given an honorary D.Sc. by the University of Princeton. After the war his services to his country were recognised by the distinction of G.B.E. which was conferred on him. During his later years he continued his interest in Economic Zoology. He was one of the founders of the Imperial College of Agriculture in Trinidad, an institution designed to give young graduates in botany some practical acquaintance with tropical plants and tropical plant disease. He crossed the Atlantic in order to enlist the support of his American friends in the project and brought back the most intriguing stories of the workings of Prohibition. He was for many years Chairman of the Council of the Marine Biological Association and took a keen interest in the application of zoology to the development of fisheries.

His Sunday evenings in the Lodge at Cambridge worthily continued the tradition of the famous Sunday evenings of Prof. Newton who had been his warm friend and who conferred on him his first University appointment.

Altogether in his death the University and the Science of Zoology have lost a most capable administrator, an enthusiastic friend of science, and one of the most loveable of men. He will long live as a gracious memory in the minds of his innumerable friends.

E. W. MacBride.

REVIEWS

Vort Landbrugs skadedyr. By Sofie Rostrup and Mathias Thomsen. Copenhagen, August Bangs Forlag, 1928. Pp. xix + 348; 224 figs.

This volume is the fourth edition of the excellent handbook of the agricultural pests found in Denmark. The third edition appeared in 1907 and has been long since sold out. Owing to the development of the applied aspect of agricultural zoology during the past 20 years, the present edition is considerably enlarged and the authors have found it necessary to re-write practically all the sections. It is a most pleasing and handy volume, the subject matter is clearly set out and the book is illustrated by 224 splendid figures of which 140 are original and 37 taken from publications of the senior author.

Mrs Rostrup has a thorough and intimate first-hand knowledge of the subject based on many years' observations and research, and during later years she has been ably assisted by Prof. Thomsen. The facts contained in this volume are therefore based on extensive field observations and experimental work, and they are presented in a manner not unduly technical, so that agricultural students, teachers and others who do not possess a special knowledge of zoology will find the book helpful and interesting.

While in the main the information is based on the conditions obtaining in Denmark, the literature of other countries has been sifted and referred to where thought desirable, and economic entomologists in other countries will find the volume is well worth a place on the book-shelf. A useful bibliography of more recent literature is given (pp. 331–41) and there is a key (pp. 305–30) to the more important insects, etc. found attacking the various crops.

The various species are dealt with under their respective classes, orders and families and include nematodes, insects, mites and other arthropods.

JAMES DAVIDSON.

Animal Biology. By J. B. S. Haldane and J. Huxley. Oxford University Press. Pp. xviii + 344. 10*s.* net. Cheap edition, 6*s.* 6*d.* net.

In the preface the authors tell us that this book is intended for that increasing section of the general public who want something more solid and more continuous than the bulk of popular scientific literature. Solid and continuous literature requires solid and continuous effort on the part of the readers and there is no doubt that, in spite of the charm and lucidity with which the book is written, anyone who takes it up without considerable preliminary biological training has a month or two of quite serious study before him. It would, however, be difficult to suggest any direction in which that effort could be more profitably spent.

An introductory chapter of 41 tightly-packed pages on the structure of a representative vertebrate, the frog, provides the necessary background for the understanding of what follows. The next chapter is on development and heredity, after which a brief account of exchanges of matter and energy leads on to five chapters on physiology, mostly from the human standpoint. The question of development is then taken up again this time from the physiological point of view. So far not much attention has been given to any phyla but the vertebrates, but the next two chapters on Evolution are of wider scope. The final chapters, in which the whole of the Animal Kingdom is passed in review in the space of 82 pages, are breathless

reading, but even among students who know their text-books well there are probably few who would not learn something new from them.

The abundant and attractive illustrations, many of which are either new or taken from not easily accessible sources, add greatly to the value of the text, and a glossary has enabled the authors to use technical terms freely without loading the text with definitions.

The basal idea of the book is to bring together into a unified science all the various specialised sections into which the study of animal life has become split. It may seem ungenerous to complain of omissions from a book so full of solid matter, but the unification can hardly be considered complete without a fuller consideration of animal behaviour than we are given here. The gap between those who call themselves "naturalists" and those who prefer to be known as "biologists" remains, and one can imagine a member of that general public for whom the book is primarily written still wondering at the end of it whether it is "done" in the critical company of highbrow "biologists" to confess an enthusiasm for, shall we say, the writings of E. T. Seton.

H. SANDON.